NEW CHROMONE ALKALOIDS FROM THE ROOT - BARK OF SCHUMANNIOPHYTON MAGNIFICUM (HANMS)

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NEW CHROMONE ALKALOIDS FROM THE ROOT-BARK OF SCHUMANNIOPHYTON MAGNIFICUM (HAIRMS)

BY

JAMES OLOYEDE ADEBOYE
B. Sc. (Hons.) · CHEM. (IBADAN)

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OF THE

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A B S T R A C T

The chemical investigations of some representative alkaloids of Rubiaceae are reviewed. The total syntheses of emetine and quinine are also reviewed while the biogenesis of anthraquinones and biosyntheses of chromones, nicotinic acid and nicotine are outlined. The bronchiodilator activities of Khellin and some chromone derivatives are compared and a brief review of the pharmacological actitivies of a few of the Rubiaceous alkaloids is given.

From the methanol extract of the root-bark of Schumanniophyton magnificum, a well known chromone, 5,7-dihydroxy-2-methylchromone (noreúgenin) 97 was isolated in addition to five alkaloids designated SRB_2, SRB_3, SRB_3', SRB_3'' and SRB_4. The constitutional formulae of two of these alkaloids, schumannificine (SRB_4) 138 and N-methylschumannificine (SRB_3) 147, have been shown to be new linear tetracyclic compounds with ring D being piperidine in nature on the basis of the chemical evidence and spectral analyses.

SRB_2 has been shown to be identical in physical and spectral properties with the product of dehydrogenation of schumannificine (SRB_4) which was named dehydroschumannificine 142.

The synthesis of dehydroschumannificine 142 was attempted. This was done in order to correlate the structure that was assigned to it with the natural alkaloids, schumannificine 138 and N-methylschumannificine 147, but only the first intermediate, 2,4,6-trihydroxynicotinophenone 146 was obtained. It was characterised by its spectral properties.

The spectral properties of SRB_3' and SRB_3'' are discussed briefly and since no conclusive work has been done on them they are tentatively assigned structures 148 & 149 respectively on the basis of their spectral data.

A C K N O W L E D G E M E N T S

I express my sincere gratitude to Prof. J.I. Okogun for his impressive supervision of this work and Dr. D.A. Okorie for co-supervising it. I am greatly indebted to them for their constant advice and encouragement in times of difficulties. I am also grateful to Prof. T.O. Bamkole, former Head of Chemistry Department for permitting me to do this research in his department.

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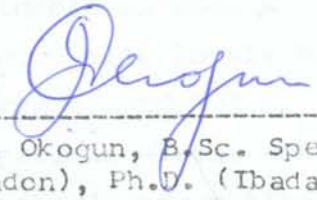
J.O. ADEBOYE

SEPTEMBER 1981.

C E R T I F I C A T I O N

We certify that this work was carried out by Mr. James Oloyede Adeboye in the Department of Chemistry, University of Ibadan, Ibadan, Nigeria.

SUPERVISORS



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I N T R O D U C T I O N

The genus Schumanniophyton (Rubiaceae) has recently attracted some attention because of the herbal medicinal use of the few species thus far classified under the genus.

Schumanniophyton magnificum (Harms) - family Rubiaceae, is a plant found and called Aide (Edo) and Ogwuakanmanu (Ibo) in Bendel State and Eastern part of Nigeria respectively and also reported in Cameroun. "It is a small tree of striking appearance with simple stem and enormous leaves. The fruits are subglobose, grey-green and covered with corky protuberances".[1] Nigerian species are reported to be up to 12ft. (3.7m) in height. A decoction of the bark is in great repute among some tribes of the Republic of Cameroun as a remedy for dysentry, and has been found effective also in the experience of Europeans.[1] Other tribes of the Republic of Cameroun use it only as a lotion after circumcision.[1] The plant is well known in the Bendel area of Nigeria for its use in the treatment of snake-bites and to scare snakes away[2], while in Eastern Nigeria the roots are used in the treatment of madness.

Rubiaceous plants are widely distributed and with the number of the genera put at eighty-five in Nigeria[3] alone in

1964, one could appreciate how huge the family is. Several genera of the Rubiaceae family have been extensively investigated. The result of the phytochemical and pharmacological investigations into the constituents of the various species is the successful isolation and characterisation of quite a great number of alkaloids and other classes of organic compounds and the establishment of the pharmacological activities of some of the compounds. A brief review of these investigations is given below.

TYPES OF EXTRACTIVES FROM RUBIACEAE.

The family Rubiaceae has been found to contain at least five groups of compounds. These include the alkaloids, anthraquinones, chromones, terpenoids and the glycosides. Of these groups of compounds, the alkaloids have attracted the greatest attention.

Although there is no comprehensive review of the distribution of the alkaloids in Rubiaceae in recent times, Raffauf, R.F.[4], in his "Handbook of alkaloids and alkaloid-containing plants" recognized the occurrence of more than 156 alkaloids belonging to different classes. These had been isolated and characterised as far back as 1970. The alkaloids mentioned below are few representatives of the

major groups: Emetine 48, with molecular formula $C_{29}H_{40}N_2O_4$ (m.p. 74°C), which forms the principal alkaloid from the roots of Psychotria ipecacuanha, and the related alkaloids, yohimbine, corynane, corynantheine, corynoxeine, mitraphylline, quinine, harman, berberine, caffeine, quinamine, cinchonamine, theobromine and a few others. In recent times, three different categories of alkaloids were isolated from Nauclea diderrichii by Mclean, S. et. al.[5], namely: β-carboline, simple pyridines and indole-pyridine alkaloids. A new pyridine derivative and two new piperidine-2-one alkaloids isolated from Schumanniophyton problematicum were reported by Schlittler, E. et al.[6]

A brief account of the chemical investigation, distribution and synthesis of

(i) pyridine and indole alkaloids

(ii) oxindole alkaloids,

(iii) pentacyclic indole bases,

(iv) corynane and heteroyohimbane alkaloids

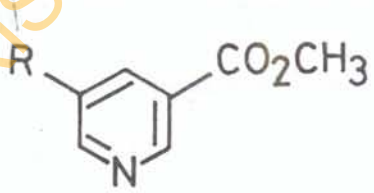
(v) piperidine-2-one alkaloids is given in this introduction. The total syntheses of emetine and quinine

are discussed in detail because of many interesting chemical reactions involved and the complexities of the molecules. The biogenesis of anthraquinones, biosyntheses of chromones, nicotinic acid and nicotine are outlined. The distribution of terpenes and the terpenoids is included as a highlight on the various groups found in Rubiaceous plants. Finally, the bronchiodilator activities of Khellin and some chromone derivatives are compared while a brief account of the pharmacological activities of a few of the alkaloids is given.

I. PYRIDINE AND INDOLE ALKALOIDS.

The pyridine alkaloids here refer to the simple pyridines and pyridine derivatives while the indole alkaloids embrace the simple β-carbolines and indole-pyridines. These two related groups of compounds occur only in a few species of Rubiaceae, so the distribution is limited.

Simple pyridine 1(a-d), simple β-carboline 2 (a-c) and indole-pyridine alkaloids 3 and 4 had been isolated from the bark of Nauclea diderrichii by Mclean, S. et. al[5]. There were earlier reports of the isolation of simple pyridine alkaloids from Rauwolfa verticulilata (Lour)[7] Bail of Hong Kong and Alstonia venenata R.Br[8]. The two species belong to the family Apocynaceae, so, the isolation of simple pyridines from N. diderrichii appeared to be the first report from Rubiaceous plants. The co-existence of both the pyridine, β-carboline and indole-pyridine alkaloids is of potential biogenetic signififance.



1

(a) R = CH_3CHOH

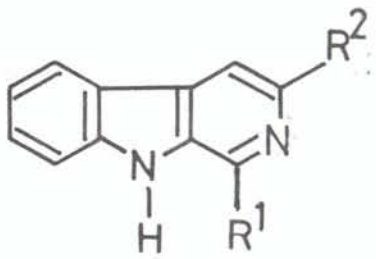
(b) R = CH_3CHOMe

(c) R = CH_3CHNH_2

(d) R = $CH_2 = CH$

(e) R = CH_3CO

(f) R = COCl



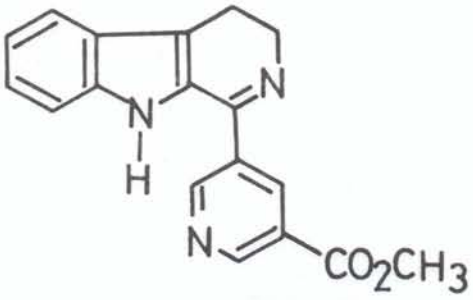
2

(a) $R^1 = CH_3$; $R^2 = H$

(b) $R^1 = CO_2Me$; $R^2 = H$

(c) $R^1 = CH_3$; $R^2 = CO_2Me$

(d) $R^1 = CONH_2$; $R^2 = H$



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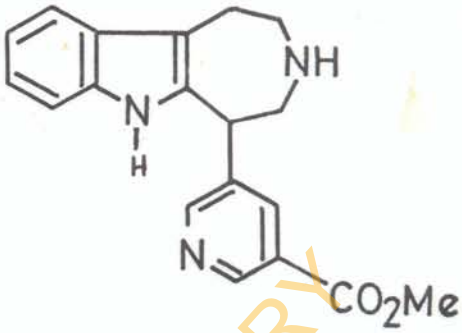
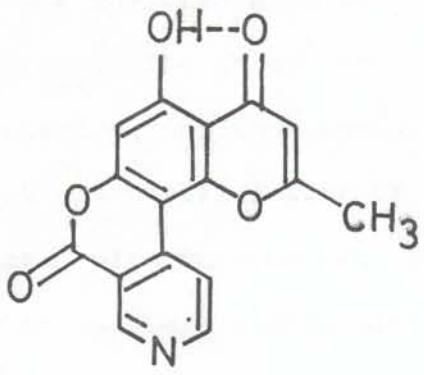


4

The question of whether pyridines are true alkaloids or artifacts produced by reaction of a precursor with ammonia used during isolation has been raised[9], because earlier procedures did employ ammonia in the extraction and chromatography steps. Doubt on this was removed when the same pyridine was obtained as in the former procedure, in a control isolation where ammonia was carefully avoided at every stage.

A new pyridine derivative, schumanniophytine 5 was isolated from the root-bark of Schumanniophyton problematicum.[6]



5

ISOLATION AND CHARACTERISATION.

The method of isolation is not the same in the two cases, but methanol extracts of the bark of N. diderrichii and root-bark of S. problematicum gave the above alkaloids. The polarity of the methanol used in the latter extraction was increased by the addition of 2% acetic acid.

In the extraction of the bark of N. diderrichii, the methanol extract obtained by soaking the coarsely ground dry

bark in methanol for 24 hours was concentrated, acidified with 5% HCl, and extracted with chloroform[10]. The aqueous phase was basified with 10% ammonia and extracted with chloroform. The chloroform extract was washed with water, concentrated, and thoroughly extracted with 5% HCl. The extract was basified with 5% ammonia and thoroughly extracted with chloroform. The final chloroform extract, after it had been washed with water, concentrated, provided the "total bases" as a brown syrup which were separated on a preparative thin layer chromatography using silica gel plates.

In the case of the pyridine derivative alkaloid 5, the dried root-bark of S. problematicum[6] was extracted with methanol containing 2% acetic acid. The soluble material was dissolved in little methylene chloride and on standing at 5-10°C for a few days gave a crystalline mixture which was chromatographed on silica gel to give the alkaloid 5 and other alkaloids.

SIMPLE PYRIDINES

The four simple pyridine alkaloids 1(a-d) were assigned structures on the basis of their spectroscopic properties

and confirmed by synthesis. In each case the molecular formula was determined from the mass spectrum; the parent ion of 1c was not observed but its formula was determined by accurate mass measurement of fragment ions (M-1 and M-15).[10]

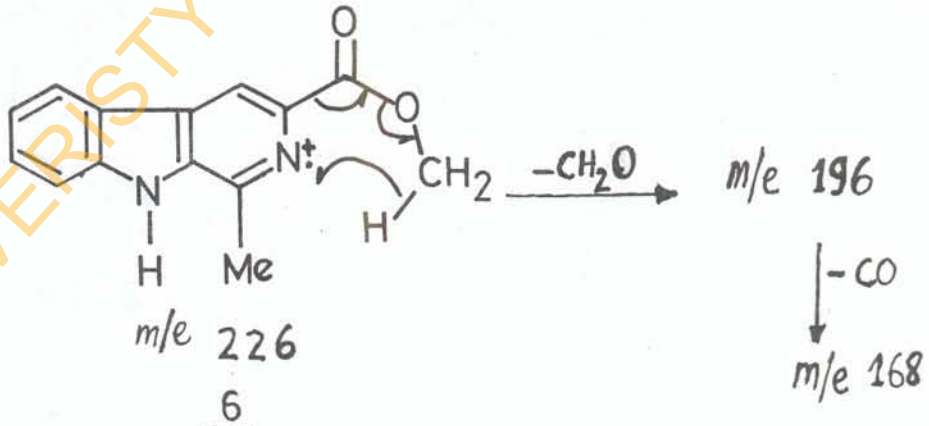
The IR spectrum of each alkaloid showed a peak at 1750cm^{-1} (with a shoulder at 1745cm^{-1}). The NMR spectrum showed a methyl singlet at δ4.0, indicative of the carbomethoxy function assigned to the alkaloid. The IR and NMR spectra also provided the evidence that led to the recognition of the structure of the side chain characteristic of each alkaloid and the NMR spectrum associated with the protons of a pyridine ring of an ABX pattern with $J_{AB} \simeq 0$ and $J_{AX} \simeq J_{BX} \simeq 2Hz$ allowed the substitution pattern of the ring to be assigned properly.[10]

SIMPLE β-CARBOLINES

The identities of the four alkaloids 2 (a-d) were easily established since they were related to a compound of known structure that is, harman 2a. They were confirmed by synthesis and compared with harman 2a. The three β-carbolines 2(a-c) had been described previously by

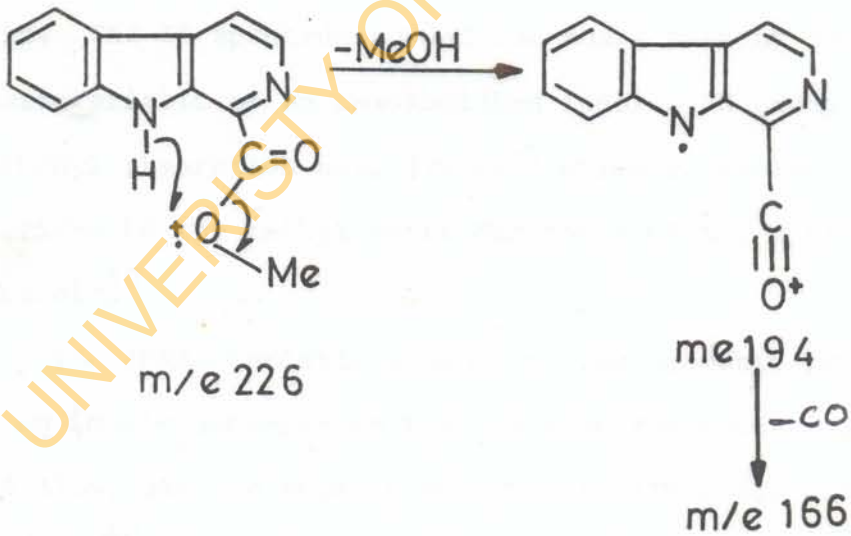
Achenbach, H. et al.[11] From one extraction, which employed ammonia 2d was isolated; this was also obtained by the reaction of synthetic 2b with ammonia and was therefore probably an artifact.

A few features of the mass spectra of these β-carbolines will be mentioned since they have some diagnostic value.[12] The base peak at M-58 in the mass spectrum of 2c did not correspond to an ion in the normal fragmentation pattern of an ester of an aromatic acid[13] and was best accounted for on the basis of a process such as shown in structure 6.



6

Evidence supporting this proposal came from accurate mass measurement and metastable ion. A corresponding fragmentation was expected for 2b and the M-58 peak was, indeed prominent; however, the base peak was at M-60 and apparently arose from a process involving the indole nitrogen as could be seen in Scheme 1. This scheme was supported by accurate mass measurement and metastable ions.



Scheme 1

INDOLE-PYRIDINES.

The indole-pyridine (naucledine) 3 will be treated as a typical example of the class since its structure has been established with certainty. The structure of 3 was deduced from the spectroscopic results and confirmed by synthesis. The formula $C_{18}H_{15}N_3O_2$ was derived from its mass spectrum[14] which showed strong parent ion, at m/e 305 and apart from a strong M-1 peak, fragment ions of low relative intensity. The complex UV. spectrum band included a prominent peak at 328nm which showed a marked bathochromic and hyperchromic change in the presence of acid. The IR spectrum showed the sharp peak at 3350cm^{-1} characteristic of an unassociated indolic NH group and carbonyl absorption near 1750cm^{-1} which resembled that ascribed to the methyl ester functions of the pyridine alkaloid.[10]

The characteristic signals of the aromatic protons of an indole appeared in the NMR spectrum between δ7.0 and δ7.8, and the signals associated with a 3,5-disubstituted pyridine[10] appeared at δ8.27, 9.15 and 9.19. The O-methyl signal was at δ3.96 and the remaining four protons produced

a symmetrical pattern of two-proton multiplets ("triplets")[14] at δ3.0 and 4.10 which resembled the pattern produced by the corresponding methylene group of oxogambirtannine.[15]

PYRIDINE DERIVATIVE ALKALOID

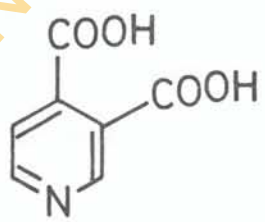
The pyridine derivative alkaloid 5 was identified from its spectra and those of its derivatives,

(a) methiodide

and (b) O-acetylderivative

The results of degradative experiments further supported the structure 5.

Oxidation of 5 with concentrated HNO_3[16] gave cinchomeronic acid 7, while treatment with strong alkali[17] gave 4,6-dihydroxy-benzo-(1',2'-2,3)-pyrano (5,4-c)-pyridine-9-one, 8



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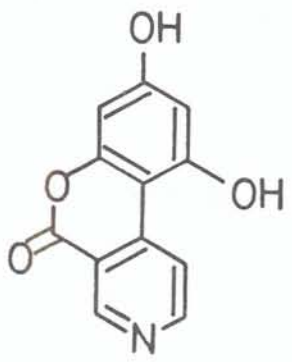

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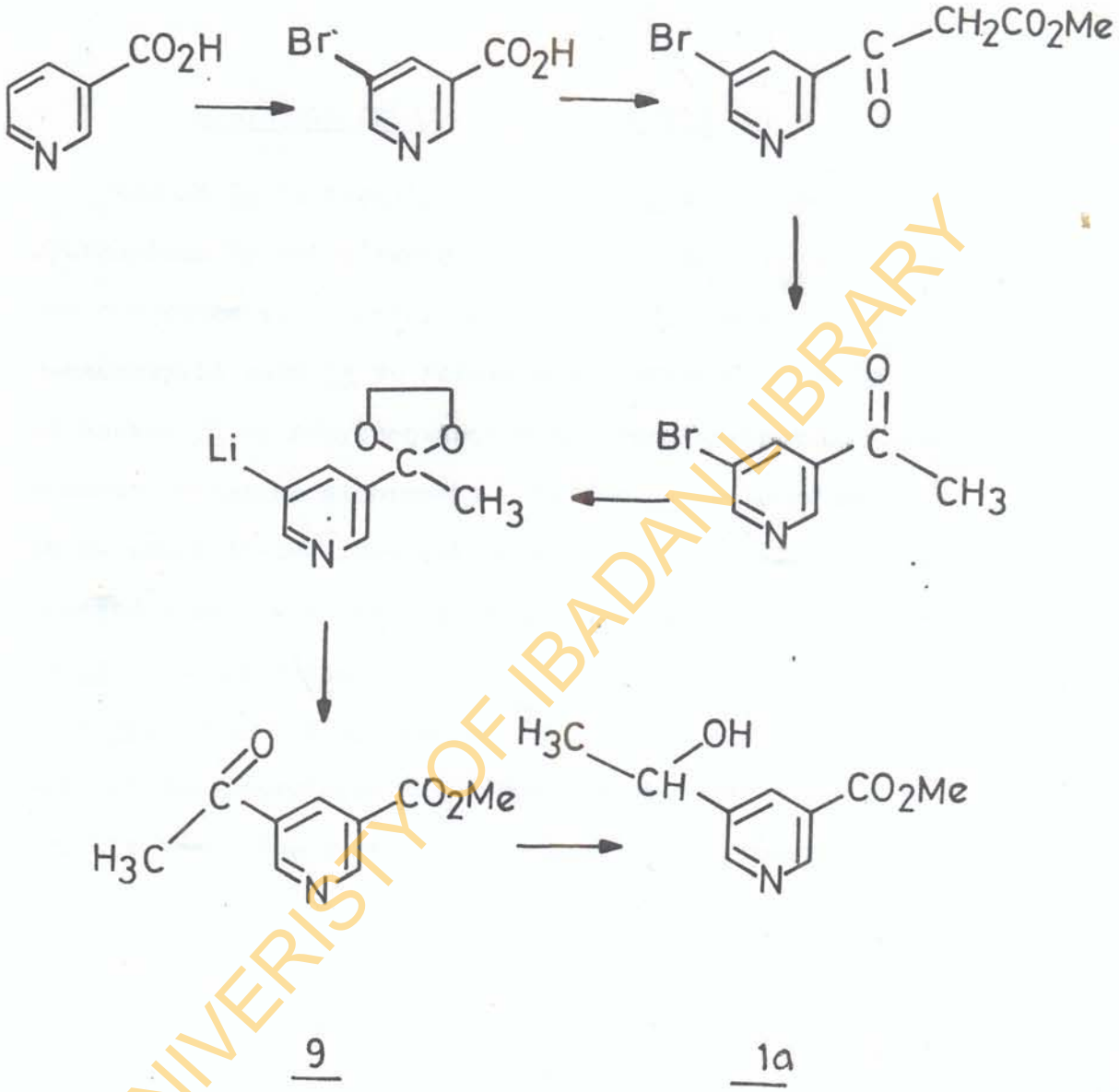
SYNTHESIS OF SIMPLE PYRIDINES

The structures of 1 (a-d) were finally established by comparisons of natural and synthetic materials. The synthetic route (Scheme 2)[10] required the preparation of the key intermediate, 3-acetyl-5-carbomethoxypyridine 9. The most successful route to 9 started with nicotinic acid which was converted through the hydrochloride of its acid chloride to 5-bromonicotinic acid; this, after esterification, was converted to the corresponding methyl-ketone by Claisen condensation.[18] The ketone was then protected as the ethylene ketal, the pyridyl lithium compound was formed, and this was converted to 9 by carbonation and esterification.

Sodium boronhydride reduced 9 to 1a. The synthetic alcohol 1a was converted by methanolysis of its mesylate to the methylether 1b. The dehydration of 1a to 1d was successfully accomplished by treatment with phosphorus pentoxide[19] or p-toluenesulphonic acid. Reduction by zinc and acetic acid of the oxime of 9 formed 1c.

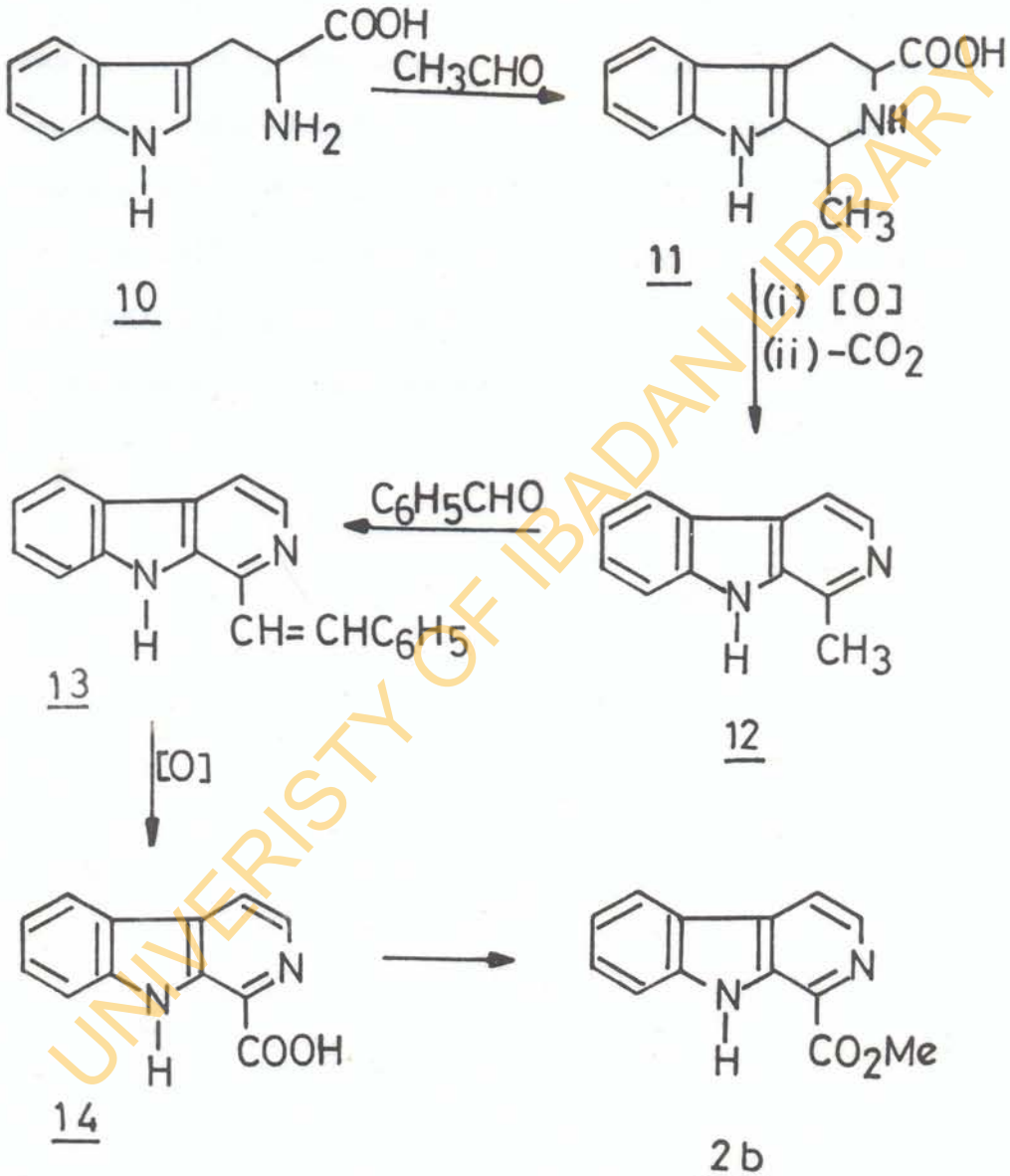


9 1a

Scheme 2

SYNTHESIS OF SIMPLE β-CARBOLINES

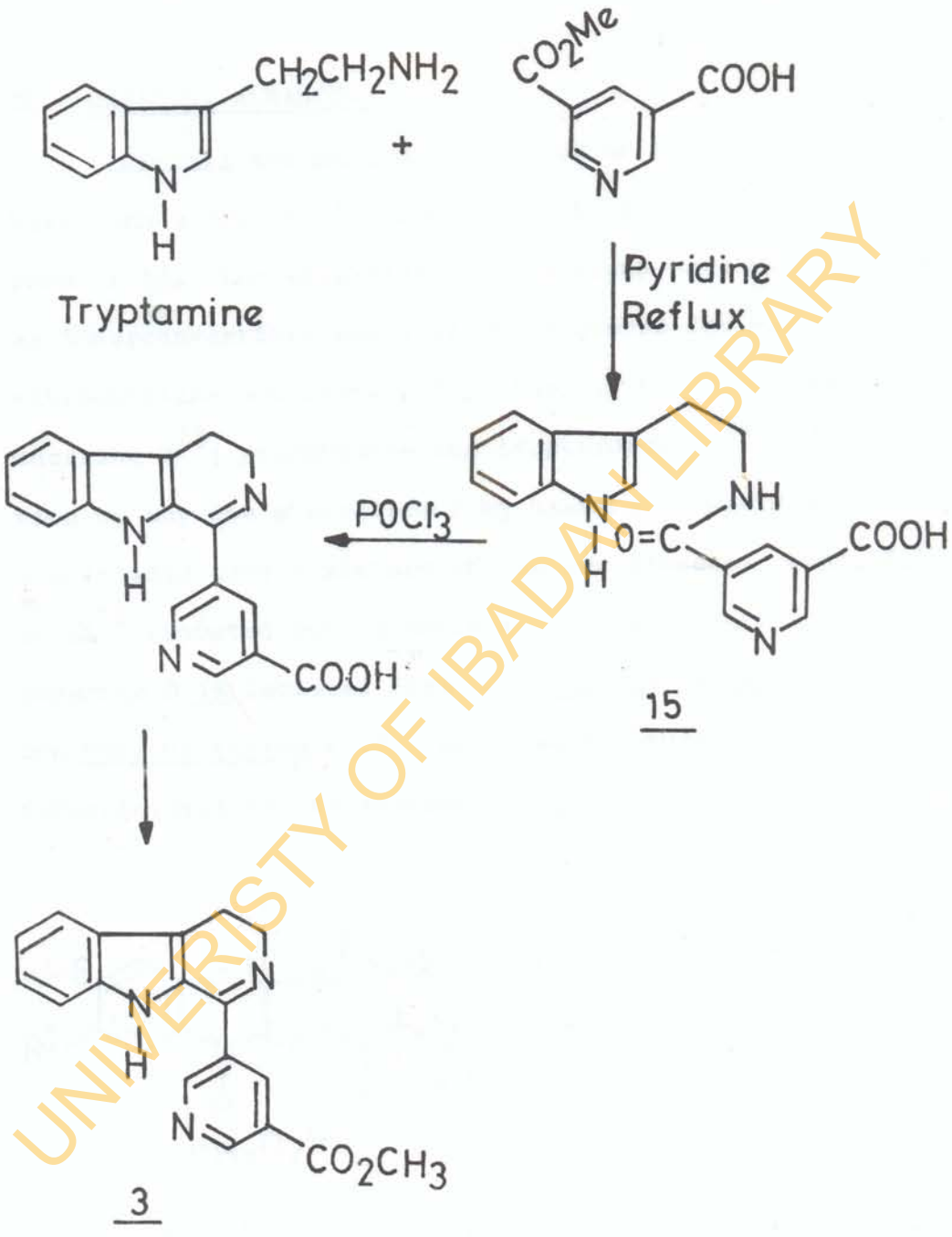
Harman 2a is readily available; 2b and 2c were synthesized by established method.[20,21] dl-Tryptophan 10 was condensed with acetaldehyde, and 1,2,3,4-tetrahydroharman-3-carboxylic acid 11 so formed was converted directly to harman 12 by dehydrogenation and decarboxylation with aqueous potassium dichromate. Harman 12 was oxidized to β-carboline-1-carboxylic acid 14 by condensing it with benzaldehyde[22] and the resulting product 13 was oxidized to 14 with potassium permanganate. Esterification of 14 gave 2b. When 1,2,3,4-tetrahydroharman-3-carboxylic acid 11 was not decarboxylated but dehydrogenated and esterified, 2c was obtained. The synthetic procedure is represented in scheme 3.



Scheme 3

SYNTHESIS OF INDOLE-PYRIDINE (Naucledine)

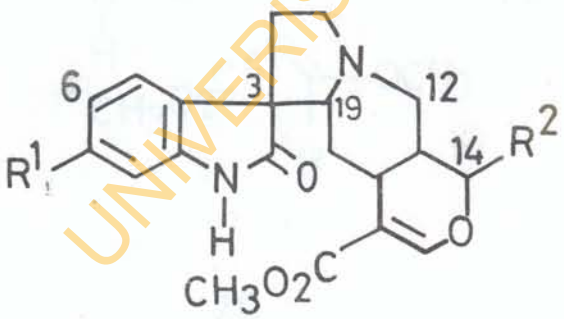
The synthesis of indole-pyridine (naucledine) 3 was effected by the reaction of the acid chloride of 5-carbomethoxy-3-pyridinecarboxylic acid (available from the synthetic work of the simple pyridine alkaloids) with tryptamine.[14] The amide 15, so formed was cyclized to naucledine 3 by treatment with phosphorous oxychloride. This is represented in Scheme 4.



Scheme 4

II. OXINDOLE ALKALOIDS

Uncaria and *Mitragyna* species of the family Rubiaceae have been a source of oxindole alkaloids having the general formula 16. The alkaloids of this group have been obtained as interconvertible pairs of stereisomers for example mitraphylline and isomitraphylline[23], uncarine A and uncarine B[23]; pteropodine and isopteropodine[24]. Equilibration of any one stereoisomer by heating in pyridine or acetic acid gave a mixture of the two isomers. John, S.R. et al[25] reported two oxindole alkaloids, uncarine C 17 and uncarine D 18 isolated from both *Uncaria bernaysii* (F.V. Muell) and *Uncaria ferrea* (D.C.) which were a further pair of interconvertible stereisomers of 16.



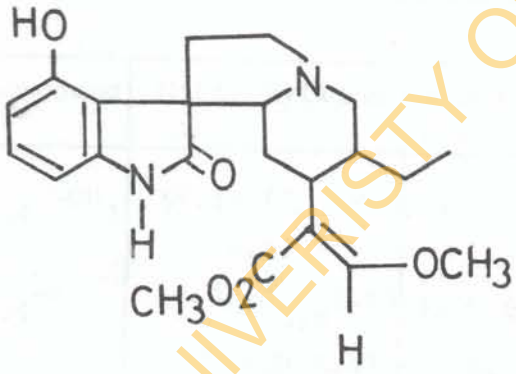
16; $R^1 = H$, $R^2 = CH_3$

17; $R^1 = CH_3$; $R^2 = H$ } Stereoisomers

18; $R^1 = CH_3$; $R^2 = H$ }

19; $R^1 = H$; $R^2 = CH_3$ (Stereoisomer of 16)

Beckett, A.H. et al.[26] reported the isolation of two oxindole alkaloids designated speciofoline 20 and "stipulatine" (rotundifoline), an isomer of 20 from the leaves of Mitragyna speciosa. A number of alkaloids were isolated from ethylacetate extracts of the leaves of Mitragyna javanica and Mitragyna hirsuta.[30] The extracts of M. javanica yielded ajmalicine, mitraphylline 19, isomitraphylline, and vineridine 21. The extracts of M. hirsuta yielded similar oxindole alkaloids.



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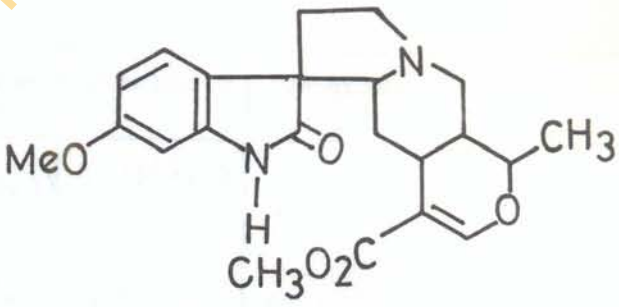

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CHARACTERIZATION AND STEREOCHEMISTRY.

Uncarine C 17 and uncarine D 18 were both shown by elemental analyses and their mass spectra to have the formula, $C_{21}H_{24}N_2O_4$, and were characterised as oxindoles by their spectroscopic properties.[25] The IR showed absorptions, ν_{max} at 1705cm^{-1} (CH_3OCO-) and 1627cm^{-1} (carbonyl), while the UV spectrum absorbed at 245nm. The NMR spectra of five of the compounds are shown in table 1.

TABLE 1.

NMR Spectra (60/Mc/s) in $CDCl_3$ solution[25]

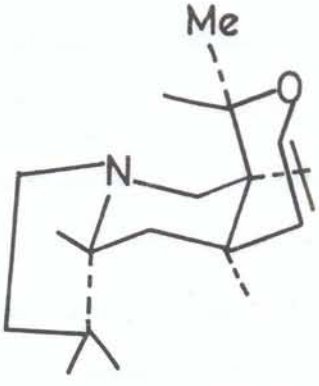
Compound	Mitraphylline	Isomitra-phylline	Uncarine B	Uncarine D	Uncarine C
C_{14} -CH_3	δ1.11 ($J_{Me,H}$ 6.4)	1.13 (6.3)	1.29(6.1)	1.22(6.5)	1.35(6.5)
C_{14}-H	δ4.34($J_{H,H}$ 2.5) ($J_{CH_3,H}$ 6.4)	4.39 (2.4, 6.3)	3.76 (2.9, 6.1)	4.15 (1.5, 6.5)	4.35 (1.2, 6.5)
$CH_3O.CO$	δ3.57	3.54	4.5	3.32	3.55
= C-H	δ7.39	7.33	7.40	7.31	7.41

Convincing evidence for the close relationship of uncarine C and uncarine D to each other and to mitraphylline 19 was obtained[27] from the mass spectra of the three alkaloids which showed a common fragmentation pattern and only minor differences in relative peak intensities.

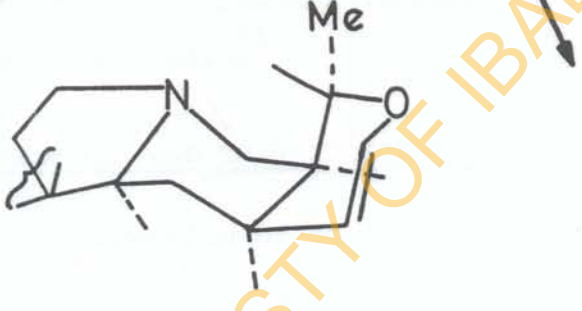
Comparison of the properties of uncarine C and uncarine D with those of the other known pairs of stereoisomers of 16 showed marked similarities, but there were, however, significant differences. Equilibration in refluxing pyridine led to the formation of almost 100% of uncarine C; while equilibration in acetic acid afforded a mixture consisting of 50% of each stereoisomer. Mitraphylline 19 and isomitraphylline have been assigned[28,29] the structure with rings DE trans and the C_{14}-H trans with respect to the C_{13}-H. Uncarine A and uncarine B were considered[28,29] to have rings DE cis and the C_{14}-H cis with respect to C_{13}-H.

Equilibration of the interconvertible isomers was considered to occur by cleavage and reformation of the C_{19} to C_3 bond.[31] Such a process could result in inversion in configuration at C_{19} and/or C_3. Because of the bulk of the group at C_{19}, Wenkert et al.[31] have proposed that isomerisation

occured with inversion of configuration at C_3 and retention at C_{19}, the C_{19}-H assuming the axial conformation with respect to ring D. Uncarine C was considered to have the DE ring junction cis and C_{14}-H and C_{13}-H trans diaxial (partial structure 22). A study of molecular models indicated that equilibration of 22 involving epimerisation at C_3 produced no marked steric interaction between the C_3-spiro group and the nitrogen atom between C_{11} and C_{13} which could produce a change in the conformation of ring D. However, if inversion at C_{19} were involved in the conversion of 22 to uncarine D, the less stable structure 23a was produced with the bulky substituent having an axial conformation. The DE cis ring junction would allow inversion of ring D to produce the more stable structure 23b (C_{19}-H axial). Such an inversion of ring D changes the conformation at C_{14} and C_{13} where the hydrogen atoms now have a trans diequatorial arrangement which is in accord[25] with the coupling constant for uncarine D. Structures 22 and 23b for uncarine C and uncarine D respectively are in accord with other evidence.

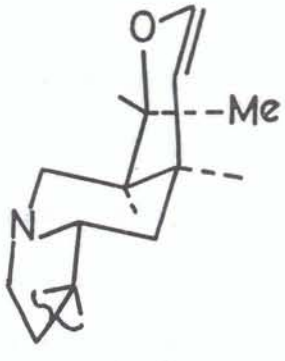


23a



22

DE CIS ; 14H ; 13H
trans diaxial



23b

DE CIS ; 14H ; 13H trans
diequatorial

III. PENTACYCLIC INDOLE BASES.

The two kinds of alkaloids that will be discussed here are the gambirtannines and yohimbines. The gambirtannines have the same skeleton with the yohimbines, but ring E of the gambirtannines are aromatized and ring D might even contain the oxo group as in the case of oxogambirtannine.

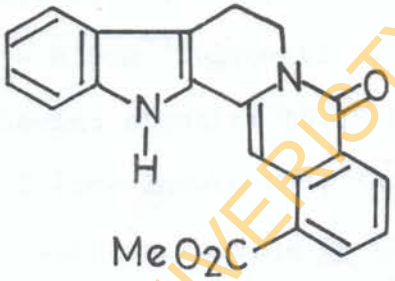
Gambir (or Gambier) is a tanning material produced by evaporation of the aqueous extract of leaves and stems of the Rubiaceae Uncaria gambier (Roxb.); a tree growing in South-East Asia.[15]

ISOLATION AND CHARACTERISATION.

Extraction of the tannin with 30% sodium hydroxide and ether gave a crude basic fraction exhibiting a strong yellow-green fluorescence. The mixture was immediately purified because it was light-and air-sensitive. Rapid chromatography through neutral alumina afforded four compounds, gambirtannine 24, dihydrogambirtannine 25, oxogambirtannine 26 and neo-oxygambirtannine 27. The structures of the compounds were characterised from their spectral properties and chemical reactions.

24

25



26

27

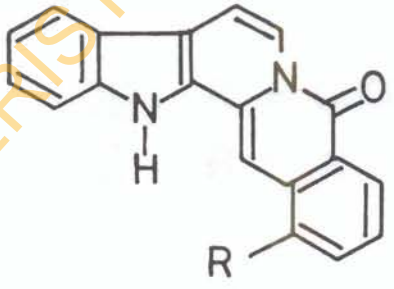
Gambirtannine 24, decomposed, even in the solid state, on standing in the air, giving rise to a small amount of oxogambirtannine 26. A solution of gambirtannine 24 in many solvents rapidly gave a mixture, from which, 24, 26 and 27 could be recovered. It was noted that 27 did not appear in the crude extract, and thus it was an antifact.

Gambirtannine 24 ($C_{21}H_{18}N_2O_2$) was optically inactive. The UV spectrum showed max at 266 (sh), 314, 340, 410nm (ε = 10500, 10840, 12800, 22400) in 95% EtOH and 250(sh) and 358nm in acidified EtOH (ε = 11300, 23000); whereas the IR spectrum (KBr) showed bands at 3340cm^{-1} (NH), 1710cm^{-1} (conjugated ester), 1590 - 1620cm^{-1} (C=C and aromatic) and 715 - 835cm^{-1} (aromatic). The NMR in $CDCl_3$ showed the following signals: δ3.10 ($\rangle CH_2-CH_2-N\langle$) δ3.89 ($-OCH_3$), δ8.55 (one indole NH), δ4.19 (2H, s, $-N-CH_2-$ aryl).

Oxogambirtannine 26 ($C_{21}H_{16}N_2O_3$), has no optical activity and has UV max at 256, 300, 346(sh), 368, 385mμ (ε = 13410, 15300, 17200, 25900, 23650) in 95% EtOH. The IR spectrum showed a complex absorption near 3340cm^{-1}, again the conjugated ester band at 1720cm^{-1}, a strong peak at 1650cm^{-1} (amide CO) and unsaturation bands at 1580-1620cm.$^{-1}$

NMR spectrum in $CDCl_3$ has the following signals; δ3.08 and δ4.49 ($\curvearrowleft CH_2(6)-CH_2(5)-N<$) , δ3.82 (3H, s, OMe) δ9.13 (-NH), δ7.99 (1H, s), δ8.30 and 8.66 (aromatic protons).

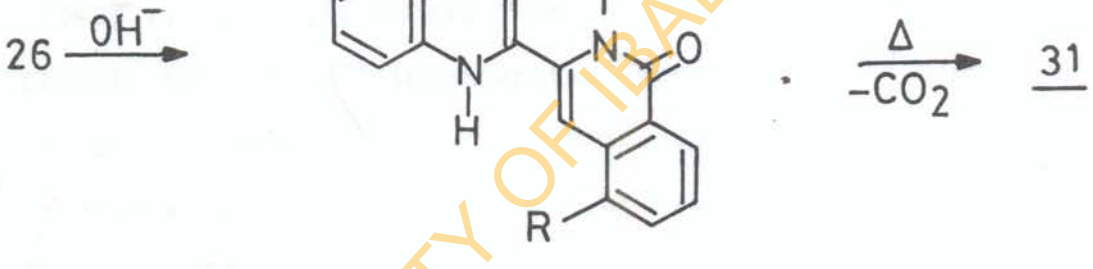
Selenium dehydrogenation of gambirtannine 24 gave a mixture of products; a strongly yellow fluorescent compound, dehydroketoyobirine 28 in low yield and nordehydroketoyobirine 29, which was also the main product of the selenium dehydrogenation of oxogambirtannine 26. The formation of both compounds in the selenium dehydrogenation of gambirtannine 24 was easily explained by assuming that the ready conversion of 24 to 26 could have occured in part before dehydrogenation.



28 ; R = CH_3

29 ; R = H

Hydrolysis of oxogambirtannine 26, gave the acid 30. Pyrolysis of the acid, 30, gave a blue fluorescent compound, norketoyobirine 31.



30 ; R = COOH

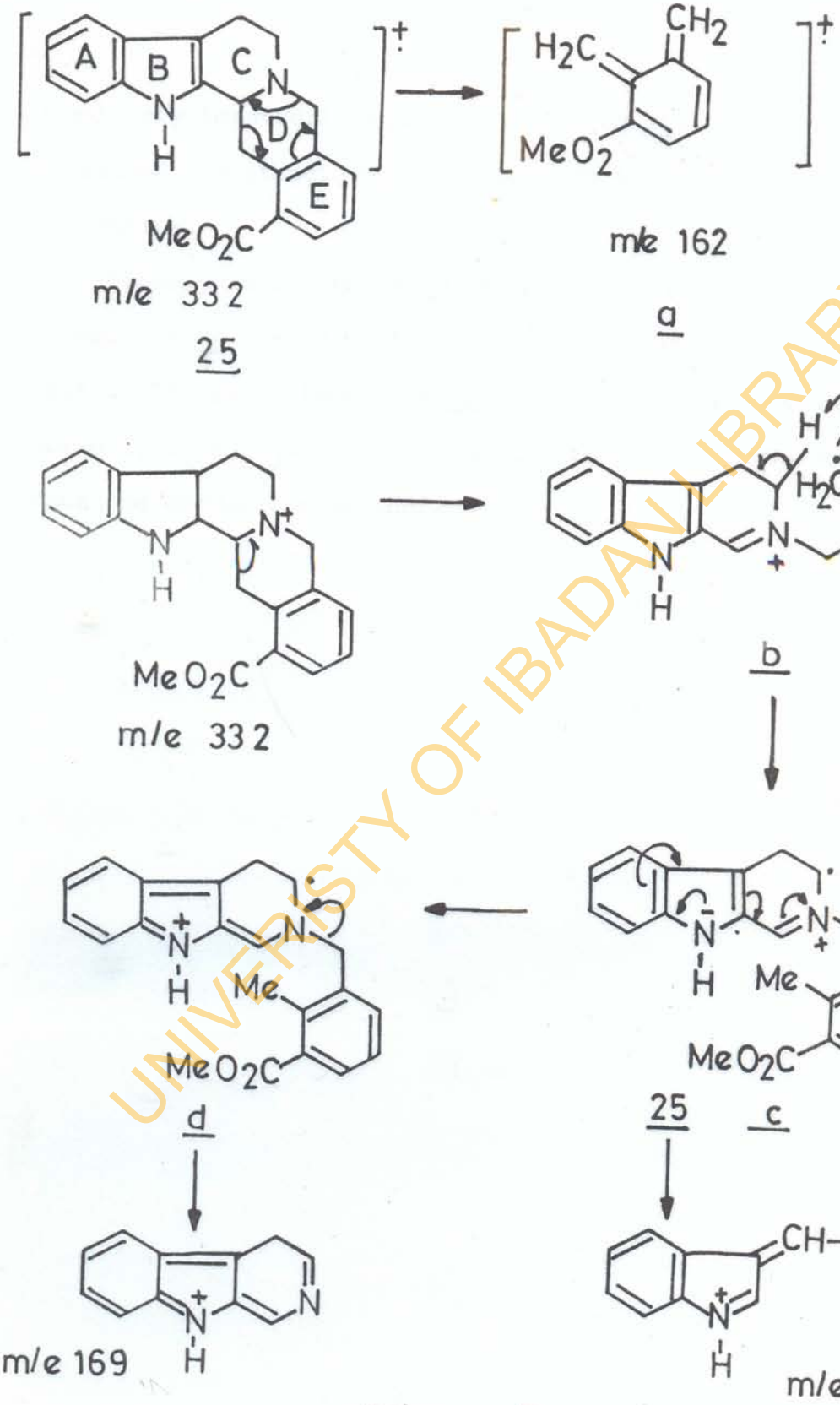
31 ; R = H

The formation of 28 from 24 during selenium dehydrogenation implied rearrangement typical of the yohimbine derivative[32], whereas the dehydrogenation of 26 which already has an amide CO, at C-21, proceeded only through the loss of the carbomethoxy group.

Dihydrogambirtannine 25 ($C_{21}H_{20}N_2O_2$), m.p. 163° gave a yellow-brown fluorescence in UV light and showed a typical unsubstituted indole chromophore (λ_{max} 225, 283, 290nm, ϵ = 60900, 12750, 11500 in 95% EtOH). The IR exhibited bands at 3340cm^{-1} (indole NH) 1705cm^{-1} (unsaturated ester) and those characteristic of aromatic absorption. The NMR spectrum (in $CDCl_3$) appeared at δ6.7 - 7.6 (integrated value of 6H.) as in compound 24, 7.84 (1H, t), 8.10 (indole NH). The complex absorption at upper field measured on integration 12H. It included the δ3.86 (OMe), the ABXY type pattern of >N-CH(3)-CH_2(14) -aryl and 2 protons at C-21.

In the mass spectrum of compound 25, which is shown in Scheme 5, a fragment ion peak at m/e 144 is characteristic for the alkaloids of this type. A retro Diels-Alder reaction in ring D gave fragment m/e 162.

Catalytic hydrogenation of gambirtannine 24 gave a corresponding compound which has its UV, IR, NMR and mass spectra



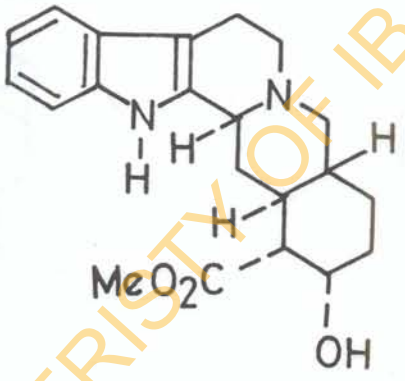
Scheme 5

completely identical with the spectra of 25. This further confirmed the structure of compound 25 to be dihydrogambirtannine.

Neo-oxygambirtannine 27 which was apparently an oxidation product of 24 showed a strongly enhanced polarity on t.l.c. plate. It was an isomer of 26, with the same molecular formula, $C_{21}H_{16}N_2O_3$. The structure was based on the spectral data and chemical reactions.

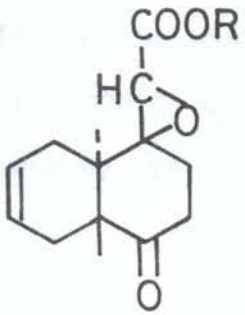
SYNTHESIS OF YOHIMBINE

The total synthesis of yohimbine 32 will be discussed as a representative of the pentacyclic indole bases. Although ring E of yohimbine 32 is not aromatized, the synthesis is of great importance in the structure proofs of some other related complex natural products.



32

Cis-Δ^6 - Octalin-1,4-dione, prepared[33] by zinc-acetic acid reduction of quinonebutadiene adduct, was converted by the Darzens reaction using chloroacetate and potassium t-butoxide, to the glycidic ester (33, R = C_2H_5) b.p. 135-155 (0.1mm). Saponification afforded a diastereoisomeric mixture of glycidic acids (33; R = H), which on heating, decarboxylated to give the unsaturated ketoaldehyde (34; R = H), b.p. 107-109° (0.01mm). Alkaline silver oxide converted the aldehyde to the acid (34; R = OH), m.p. 145-146°C.



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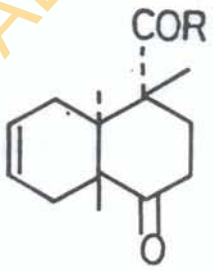

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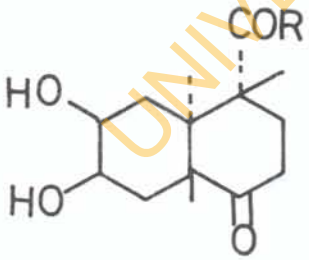

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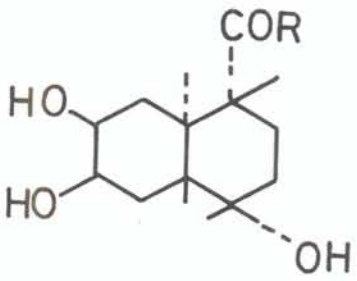
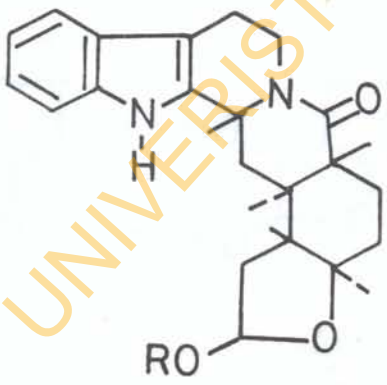


36

Treatment of the keto acid (34,R = OH) with oxalyl chloride yielded the corresponding acid chloride, which, without isolation, was used to acylate tryptamine, giving the amide (34; R = β-ind-CH_2CH_2NH), m.p. 161-162. Hydroxylation with osmium tetroxide provided the keto diol (35; R = β-ind-CH_2CH_2NH), m.p. 213-214° ; which on platinum-catalyzed hydrogenation, yielded the triol (36; R = β - ind-CH_2CH_2NH), m.p. 227-228°C. Glycol cleavage of the triol to the aldehyde (not isolated), followed by cyclization to the hexacyclic lactol lactam (37; R=H), m.p. 218-220°C. decomp.), was achieved by periodate oxidation followed by brief heating with dilute phosphoric acid.



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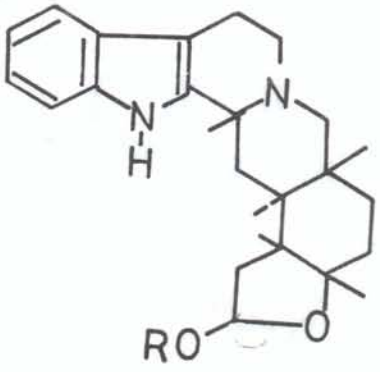
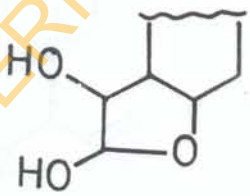


38

Acid-catalyzed methanolysis to the lactol ether lactam (37; R = CH_3), m.p. 268-270°C, preceeded lithium aluminium hydride reduction, which gave the lactol ether base (38; R = CH_3), m.p. 133 - 137°C.

The acetic acid salt of the o-acetate (38; R = CH_3CO), on brief heating at 280-290°C (in vacuo), afforded a sublimate, the acetate salt of the enol ether 39. Osmium tetroxide hydroxylation gave the expected diol 40, which was cleaved, on treatment with metaperiodate, to the o-formate of dl-pseudoyohimbaldehyde. Chromic acid oxidation of the aldehyde, carried out in methanol-acetone in the presence of sulphuric acid gave rise to dl-pseudoyohimbine 41. The pseudoyohimbine was epimerized to yohimbine 32.

39



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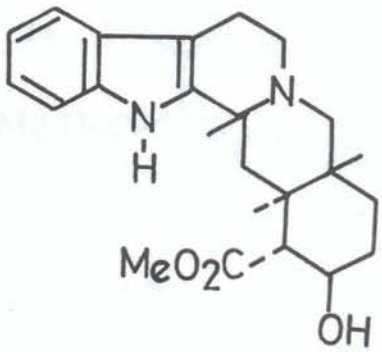
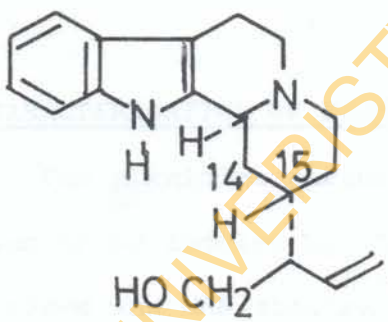


41

IV CORYNANE AND HETEROYOHIMBANE ALKALOIDS.

These two types of alkaloids are indole bases and the differences lie in the absence of ring E in the Corynane alkaloids and in the nature of the substituents. The heteroyohimbane alkaloids have structures similar to that of yohimbine 32 except for the hetero oxygen atom present in ring E of heteroyohimbane alkaloids.

Antirhine 42, the major base isolated from the leaves of Antirhea putaminosa (F.V. Muell) Bair (Rubiaceae)[34] may be regarded as the parent member of the small group of indole alkaloids which possess a 15β-hydrogen. The extracts of Mitragyna hirsuta[30] yielded hirsutine 43



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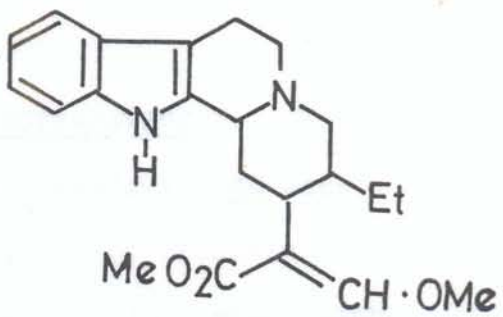


43

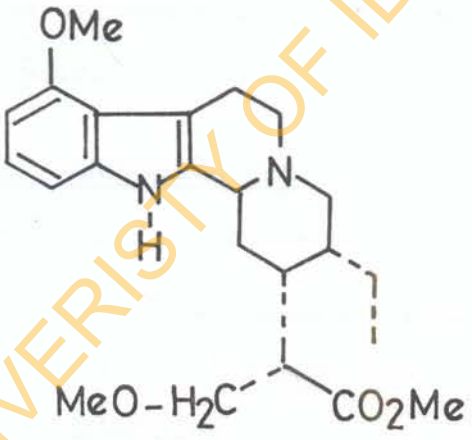
Mitrajavine, (heteroyohimhane alkaloid), 44 was isolated from the ethyl acetate extracts of the leaves of Mitragyna javanica.[30]

44

CHARACTERISATION OF HIRSUTINE AND MITRAJAVINE

The alkaloids hirsutine 43 and mitrajavine 44 were shown to be indoles by colour tests, and by ultraviolet, infrared and NMR spectra.[30] The physical data, along with elemental analysis and equivalent weight determinations indicated the structure of mitrajavine to be 44. The closed ring E was indicated by the presence of a three-proton

doublet for the C_{19} Me (δ0.90) and the C_{19}-H one proton multiplets at about δ4.40. A cis C_3H orientation was indicated by the absence of any trans C-H bands (below 2800cm^{-1}, KCl disc) in the IR spectrum and the presence of a one-proton multiplet in the NMR spectrum at δ4.45. The aromatic splitting pattern of mitrajavine 44 (δ6.53, 1H; δ7.09, 2H) is similar to that of mitragynine 45 (δ6.48, 1H; δ6.92, 2H) showing that the aromatic methoxy group (δ3.90) is in the 9-position.

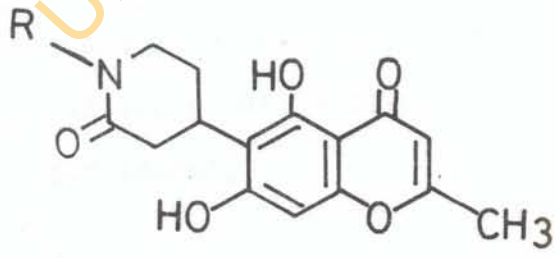


45

The physical data, along with elemental analysis and equivalent weight determinations indicated the structure of hirsutine to be of the corynantheidine type 43. The methyl of the ethyl group was indicated by the three-proton triplets at δ0.79 in the NMR spectrum. The ester and methoxy groups appeared at δ3.71 and δ3.78. A cis C_3H was demonstrated by the absence of trans C-H bands (below 2800cm^{-1}, KCl disc) in the infrared spectrum and a one-proton cis C_3H multiplet in the NMR spectrum at δ4.45. This evidence suggested that hirsutine is an alkaloid of the corynantheidine type with C_3H cis to the nitrogen lone pair.

V. PIPERIDINE-2-ONE ALKALOIDS.

Piperidine-2-one alkaloids with structures 46 and 47 were isolated from Schumanniophyton problematicum by Schlittler, E. et al[6]. This appeared to be the first report of this group of alkaloids.



46 ; R = H

47 ; R = CH_3

CHARACTERISATION

The structures of compounds 46 and 47 were proposed from their spectral properties and by comparison with the spectra of 5,7-dihydroxy-2-methylchromone. Compound 46, m.p. 303-3°C (recryst. from EtOH), has from the mass spectrum, M^+ 289; gave on methylation with diazomethane, the monoether, $C_{16}H_{17}NO_5$, m.p. 262-3°C. Only the 7-hydroxyl was methylated leaving the hydrogen-bonded hydroxyl group at 5-position unchanged. The spectral data for compound 46 are shown below.

UV λ_{max} (nm) in MeOH.

205 (log ε = 4.35), 225(log ε = 4.18), 251 (log ε = 4.25), 257 (log ε = 4.27), 295(log ε = 3.78) and 318 (log ε =3.68)

IR (KBr), (cm^{-1}), 3370 (NH), 3300~2400 (-OH)

1670 (>C=O, γ - pyranone ring and sec. amide),

1625 (>C=C<), 1600 (aromatic, >C=C<).

^{1}H-NMR δ(ppm) (d_6-DMSO).

1.6-3.8 (7 aliph. H), 2.38 (allyl CH_3), 6.16 (vinyl H), 6.3 (arom. H), 7.53 (NH), 10.88 (7-OH), 12.9 (5-OH).

MS. m/e (rel. ab. %), M^+ 289 (88), 272 (79),
245 (64), 244 (43), 231(40), 229(22),
219 (26), 218 (41), 217 (26), 216 (41),
205 (100-base peak), 193 (24), 192 (41), 189 (21).

Compound 47, m.p. 309-313°C (recry. from EtOH/Benzene) has from the mass spectrum M^+ 303 and occured in minute amount. It has the following spectra data.

UV λ_{max}(nm) in MeOH.
205 (log ε = 4.35), 225 (log ε = 4.16), 251 (log ε = 4.22)
257 (log ε = 4.23), 295 (log ε = 3.77), 318 (log ε = 3.66)

IR (KBr), cm^{-1}), 3400~2400 (OH), 2950 ($N-CH_3$)
1670 (>C=O, γ - pyranone ring and tert. amide)
1620 (>C=C<), 1600 (aromatic, >C=C<)

^{1}H-NMR (δppm) (d_6-DMSO).
1.6-3.8, (aliph. H), 2.36 (allyl. CH_3), 2.84 ($N-CH_3$),
6.14(vinyl H), 6.26 (arom. H), 10.89 (7-OH),
12.9 (5 - OH).

MS. m/e (rel. ab. %). M^+ 303 (57), 272(13),
245 (19), 244 (10) , 231 (19), 218 (24),
217 (14), 216 (17), 205 (100- base peak),
193 (16), 192 (43).

There were no degradative and synthetic works reported for both compounds.

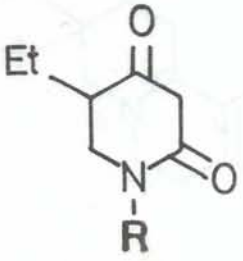
VI. SYNTHESIS OF EMETINE AND QUININE.

a. EMETINE

There has been much interest in the synthesis of emetine[35-36], and all routes used yielded mixture of stereoisomers. With the stereochemistry of emetine 48. known, a controlled synthesis was reported.[37]

48

The readily prepared oxopiperidone 49 was reduced catalytically or by boronhydride to the mixture of epimeric alcohols 50. Acetylation of the mixture followed by elimination of acetic acid over hot sodium acetate yielded the 5,6-dihydropyridone 51. Michael addition of diethylmalonate anion to compound 51 occured with steric control, since the product {52; R'=$CH(CO_2Et)_2$}, by hydrolysis and decarboxylation yielded over 76% of the desired trans acid (52, R'=CH_2·COOH), m.p. 153-153.5°C.



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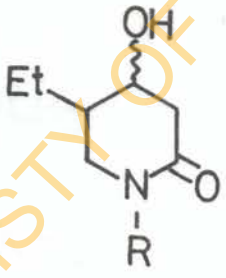

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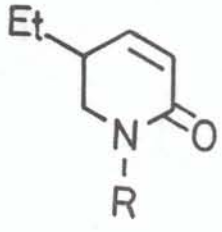

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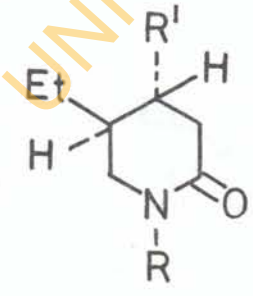

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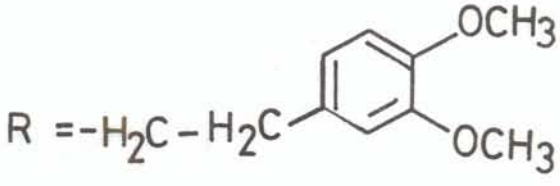
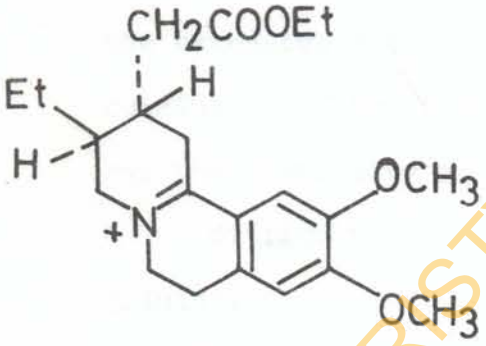


This, as its ethyl ester, was cyclised by phosphorus oxychloride to compound 53 isolated as the perchlorate, m.p. 113-114°C, which on catalytic hydrogenation gave 90% of the base, 54, m.p. 66-66.5°C. This base has its three asymmetric centres orientated as in emetine. The amino acid derived from compound 54, was converted into its homoveratrylamide 55, mp. 146.5 - 147.5°C, using the mixed anhydride method.



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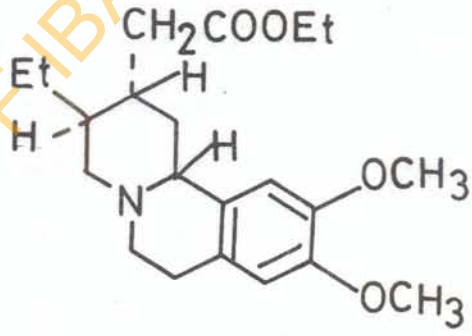

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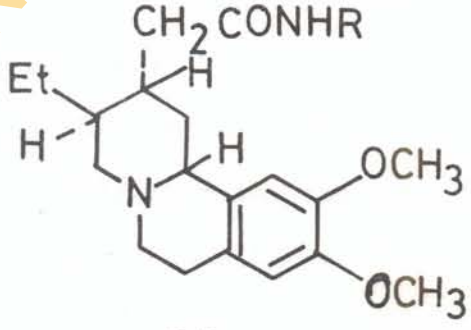


55

Phosphorus oxychloride cyclised compound 55 to yield DL-O-methylpsychotrine,as 48, but with double bond between C_1 and N, which was resolved as its dibenzoyl tartarate. Catalytic reduction of the resultant, (+) -O-methylpsychotrine, m.p. 122.5 - 123.5, yielded (-) - emetine, isolated as its hydrobromide and further characterised as its N-benzoyl derivatives, m.p. 183.5 - 184.5°C.

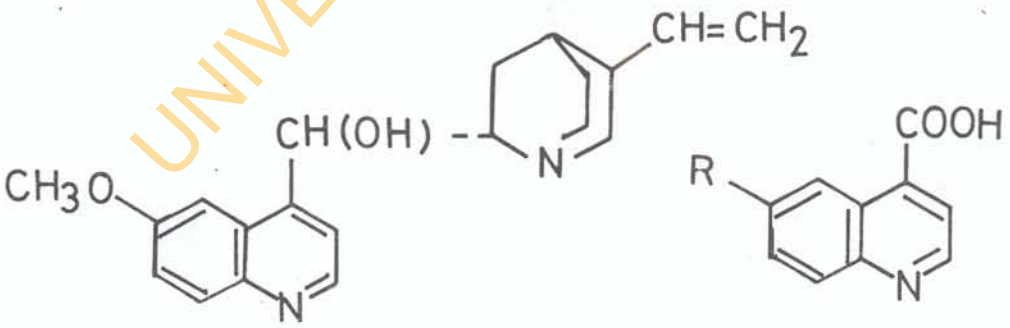
b. QUININE.

The pure crystalline quinine was isolated in 1820 and the extensive degradative researches of the last century culminated in the proposal of the correct structure in 1908, but the complexity of the molecule has placed some serious difficulties in the way of the total synthesis of quinine.

The synthetic investigations which were undertaken shortly after the turn of the century culminated[38] in the total synthesis of dihydroquinine in 1931 and that of quinine 56 in 1944. The first great advance was made when it was shown that the cinchona bases could be prepared by partial synthesis from the toxines, which contain two fewer asymmetric centres than the alkaloids themselves. This

discovery focussed attention on the development of methods for the synthesis of quinoline 4-ketones from components representing the quinoline and quinuclidine portions of the alkaloid molecules. The synthesis of substances useful in the introduction of the quinoline moiety was accomplished during the early phases of the synthetic studies. The developments along these lines set the stage for the final solutions of the synthetic problem, which were achieved with the elaboration of methods for the synthesis of suitable components for the incorporation of the quinuclidine residue.

The starting point for all the syntheses of the cinchona bases and related substances was the quinoline portion of the molecule, represented by cinchoninic acid (57; R=H) or quininic acid (57; R = OCH_3)



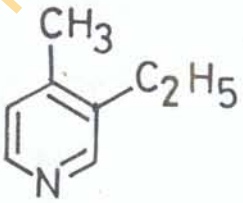
56

Cinchoninic acid, 57; R= H
Quininic acid, 57; R = OCH_3

Cinchoninic acid has been obtained from isatin and acetaldoxine[38]; by decarboxylation of quinoline-2,4-dicarboxylic acid obtained by condensation of isatinic acid and pyruvic acid) and by oxidation of lepidine.[38-40] There are many other interesting synthetic methods that lead to the quinoline portion that are not mentioned here.

The synthetic investigations related to the quinuclidine 58 moiety had as their first objectives the synthesis of simple quinuclidine derivatives and degradation products from the alkaloids.

58



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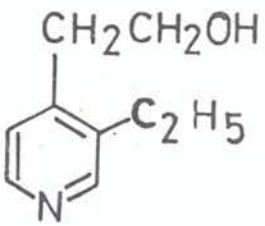
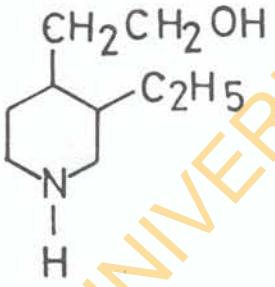


60

In 1904, Koenings[38] showed that β-collidine 59, which was at that time available only from the degradation of cincholine could be condensed with formaldehyde to give 3-ethyl-4-(β-hydroxyethyl)-pyridine 60, which on reduction with sodium and alcohol yielded the corresponding hexahydro derivative 61. The latter substance was converted by treatment with hydroiodic acid and phosphorus into an iodo compound 62, which was stable only as its salt, and which was transformed into the hydroiodide of 3-ethylquinuclidine 63 on standing in ether solution.



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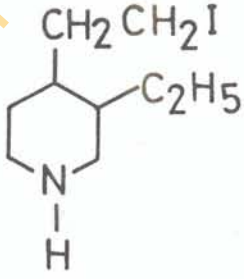

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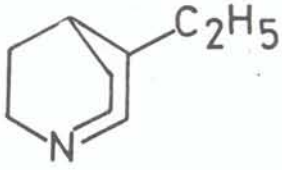
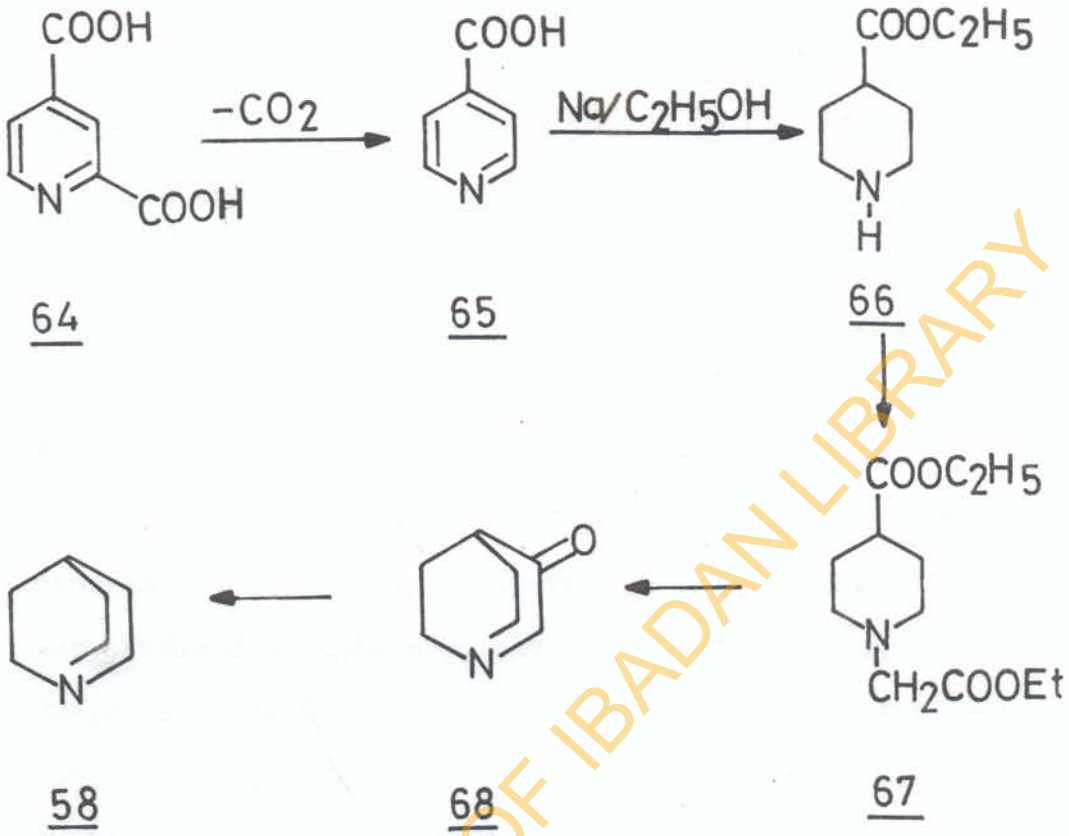


63

A further synthesis of quinuclidine was devised by Clemo and Metcalfe[41,42], who decarboxylated 2,4-lutidinic acid 64 obtained by oxidation of 2,4-lutidine and reduced the resulting pyridine-4-carboxylic acid 65 with sodium and alcohol. Esterification of the product afforded ethylpiperidine-4- carboxylate 66, which was condensed with ethyl chloroacetate to give ethylpiperidine-1-acetate-4-carboxylate 67. Dieckmann cyclisation followed by decarboxylation yielded 3-ketoquinuclidine 68, which on reduction by Wolff-Kishner or Clemmenson methods gave quinuclidine. These are shown in Scheme 6.



Scheme 6

TOTAL SYNTHESIS OF QUININE

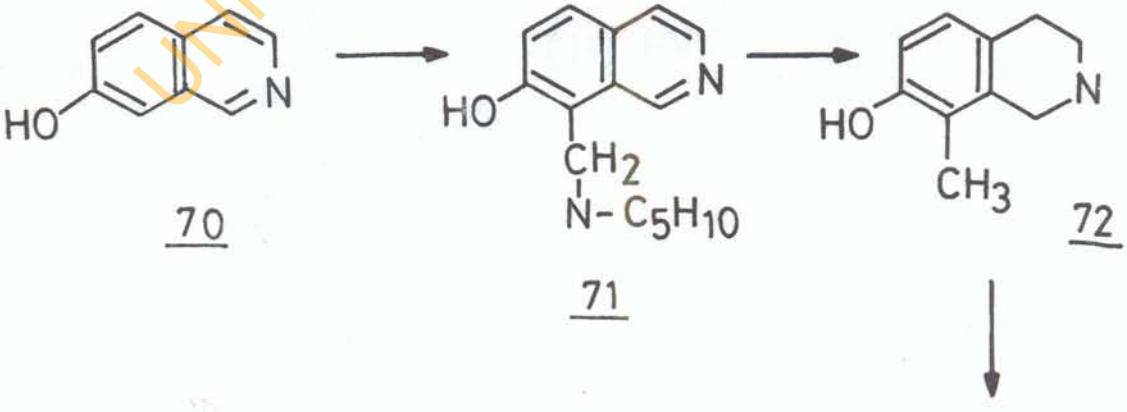
Through the investigations described above; the problem of the total synthesis of quinine had been reduced

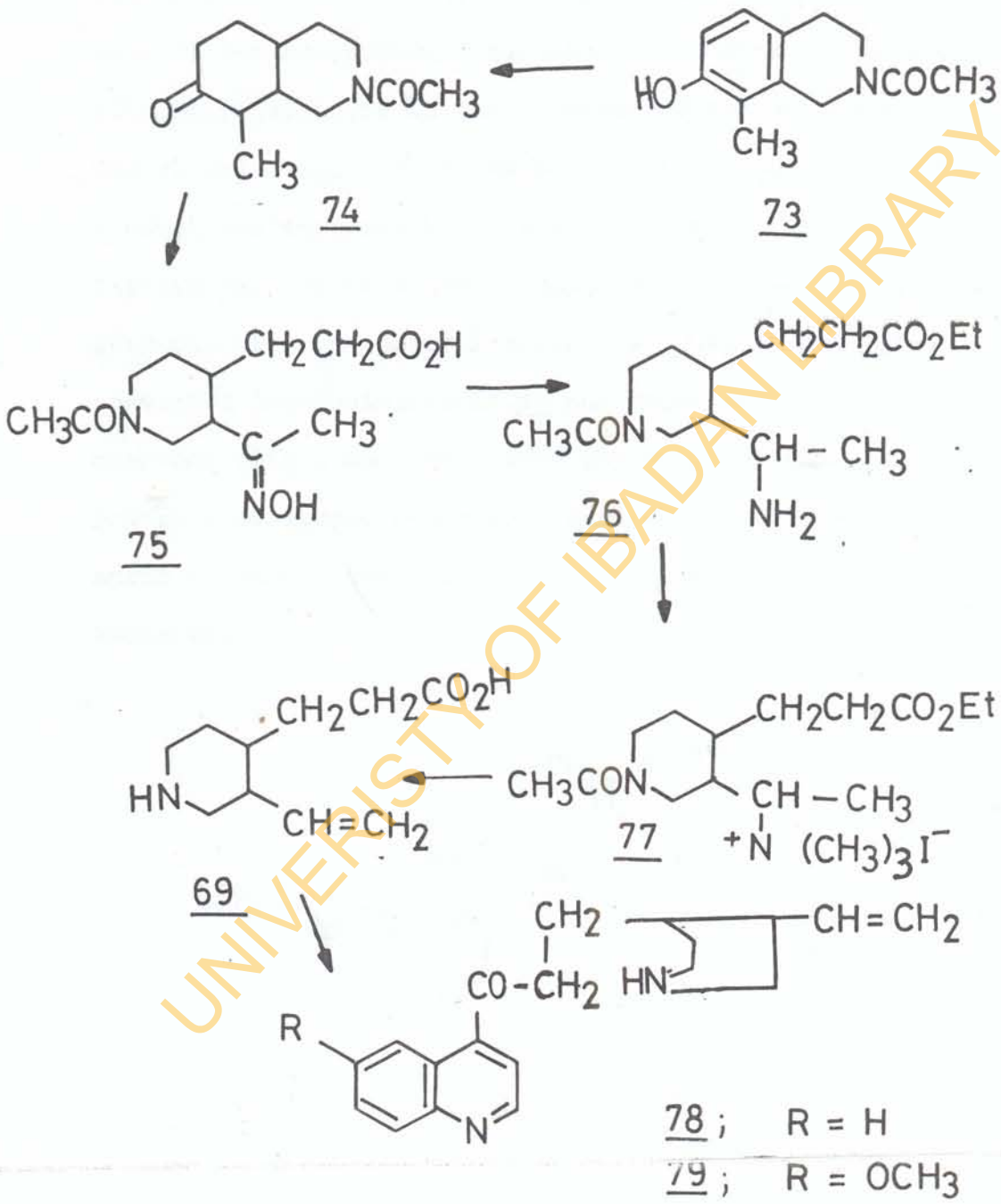
to that of the synthesis of homomeroquinene 69. This goal was reached[43] in 1944.

The starting point of the synthetic work was 7-hydroxy-isoquinoline 70 which was obtained by condensation of m-hydroxybenzaldehyde and amino acetal, followed by cyclisation with sulphuric acid. The carbon atom required for completion of the homomeroquinene skeleton was introduced by condensation of 70 with formaldehyde and piperidine. 7-Hydroxy-8-piperidinomethylisoquinoline 71 was obtained in this way, and was smoothly converted into 7-hydroxy-8-methylisoquinoline 72 by treatment with methanolic sodium methoxide at 220°C. On catalytic hydrogenation in acetic acid solution, 72 absorbed two moles of hydrogen and after acetylation with acetic anhydride in methanol furnished an N-acetyltetrahydroderivative 73. High pressure reduction of 73 over Raney nickel afforded a mixture of stereoisomeric N-acetyl-7-hydroxy-8-methyldecahydroisoquinolines. Direct oxidation of the crude hydrogenation product yielded a mixture of ketones 74 and (the trans isomer) from which the cis form 74 was separated as a crystalline hydrate. Rupture of the carbocylic ring was brought about by sodium ethoxide and

ethyl nitrite, and furnished N-acetyl-10-oximinodihydrohomomero-quinene ethyl ester 75. The oximino-ester was then hydrogenated to the corresponding amino-ester 76.

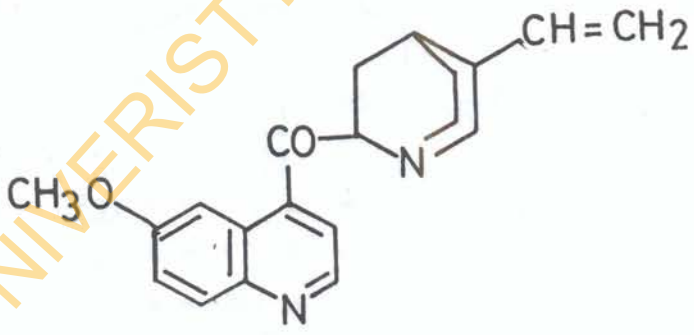
N-Acetyl-10-aminodihydrohomomeroquinene ethyl ester 76 was converted to N-acetyl-10-trimethylammoniumdihydrohomo-meroquinene ethyl ester iodide 77 by treatment with methyl iodide and potassium carbonate. With 60% potassium hydroxide elimination occured according to the Hofmann rule and after treatment with potassium cyanate, homomeroquinene 69 was isolated from the reaction mixture as the N-uramido derivative. Regeneration of the homomeroquinene was accomplished by hydrolysis with dilute acid, and in this way, cis-dl-homomero-quinene was obtained. The whole synthetic procedure is represented in scheme 7.





Scheme 7

Resolution into optically active forms was not attempted at this point, but the product was converted into dl-N-benzoylhomomeroquinene ethyl ester and condensed with ethylquininate in the presence of sodium ethoxide. The dl-quinotoxine obtained on hydrolysis of the condensation product was resolved by crystallization of the dibenzoyl-d-tartarates, and furnished d-quinotoxine, identical in all respects with the natural material. Quinotoxine 79 was converted into quinidinone 80 and reduction of the latter compound with aluminium powder and ethyl alcohol in the presence of sodium ethoxide afforded a mixture of stereoisomeric alcohols from which quinine 56 and quinidine were isolated.

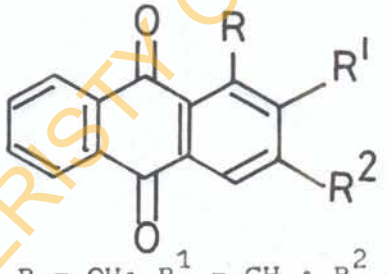


80

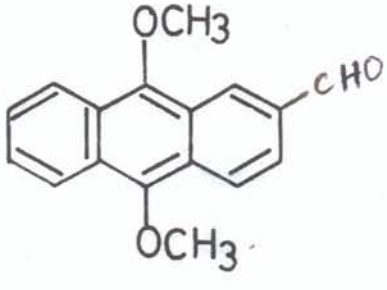
VII OCCURENCE AND BIOGENESIS OF ANTHRAQUINONES.

(a) OCCURENCE

The anthraquinones, 1-hydroxy-2-methylanthraquinone 81, 3-hydroxy-2-methylanthraquinone 82 and quite a number of others whose biogenetic relationship has been well documented[44], occur in Rubiaceae. 4-methoxy-1-naphthol has been found in Rubiaceous plant.[45] The anthraquinols, oruwal 84, its 5- or 8- hydroxyderivative oruwalol and 10-anthraquinones, have been isolated from the stem of Morinda lucida (Rubiaceae).[46]



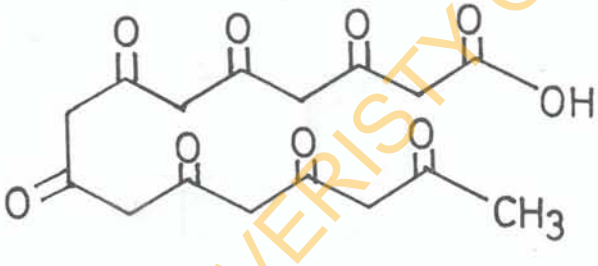
81; R = OH; R^1 = CH_3; R^2 = H
82; R = H ; R^1 = CH_3; R^2 = OH
83; R = OH; R^1 = H; R^2 = CH_3.



84

(b) BIOGENESIS.

In contrast to the anthraquinones of the emodin 83 type which were considered to arise by suitable folding and condensation of a polyketide chain derived from eight acetate units as 85, about half of those found in higher plants are substituted in only one benzenoid ring and may be totally devoid of a carbon side chain or hydroxyl groups, e.g. 86 and 87 respectively. Majority of these occur in Rubiaceae sub-family (Rubiodeae) and to lesser extent in the Bignoniaceae and Verbenaceae; tectoquinone 87 being present in all the three.[44]



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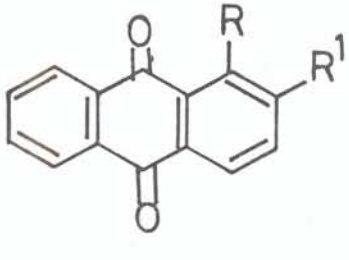
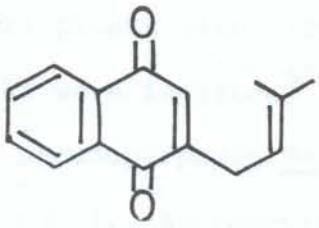


86 ; R = OH ; R′ = OH

87 ; R = H ; R¹ = CH_3

Significantly the anthraquinones present in Biognoniaceae[47,48] and Verbenaceae[44,49] heartwoods are all accompanied by C_{15} naphthaquinones notably deoxylapachol 88, while the Rubiaceous plants contain a number of C_{15} naphthalenic compounds represented by 89, 90 and 91.



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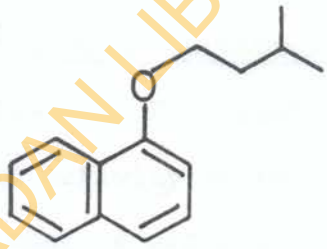

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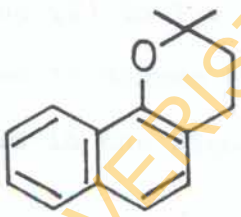

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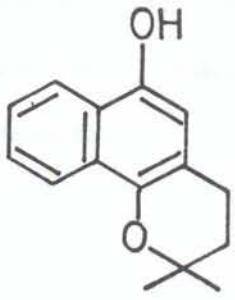
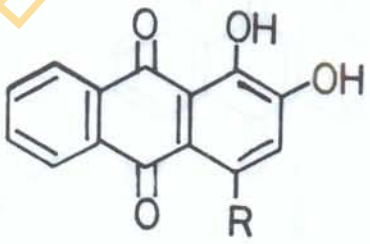


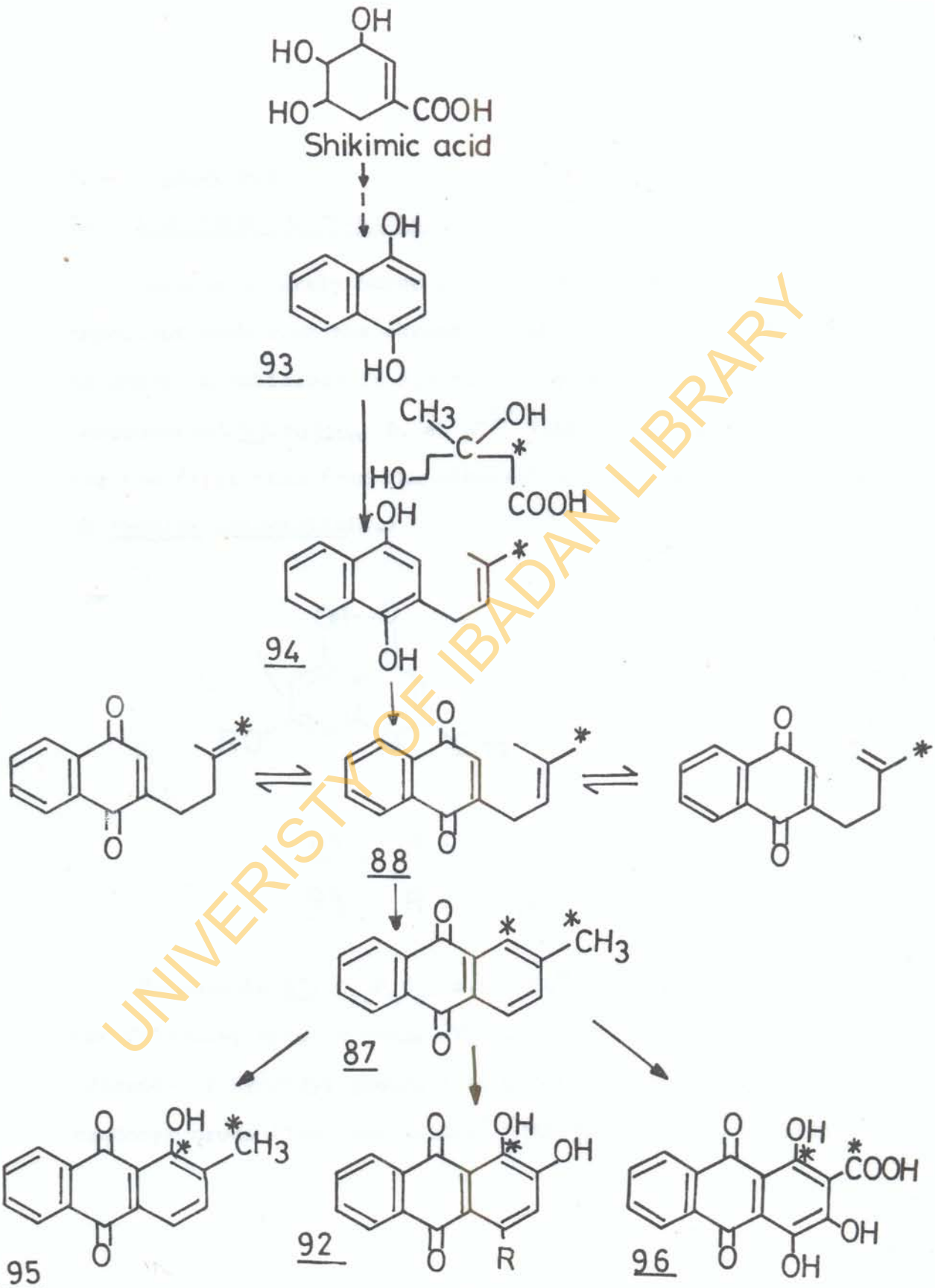
91

These findings suggested that 88 was synthesized in vivo by prenylation of a naphthol precursor followed by oxidation and since 88 could also be converted to 87 in vitro[47], either by borontrifluoride catalysis or by irradiation, it seemed likely that the substituted (C) ring in this group of anthraquinones was derived from mevalonate. This was established[49,50,51] by feeding Rubia tinctorum (madder) plants with {2-^{14}C} - mevalonate. Four radioactive pigments were isolated[49], the specific activity of rubiadin 95 and pseudopurpurin 96 being twice that of alizarin (92; R = H). Appropriate degradation of pseudopurpurin 96 established that the ^{14}carbon was distributed between the side chain and C_1 in ring (C). It seems therefore that the ring (C) in the Rubiaceous anthraquinones is formed as shown in Scheme 8 and presumably the same pathway is followed in the Bignoniaceae and Verbenaceae.



92

Alizarin (92 ; R = H)
Purpurin (92 ; R = OH)

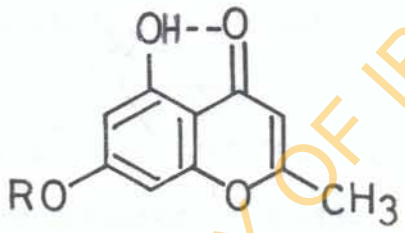


Scheme 8

VIII. CHROMONES.

(a) ISOLATION AND CHARACTERISATION.

Chromones rarely occur in Rubiaceous plants. The only important member of the chromones, which has been reported to occur in Rubiaceae is 5,7-dihydroxy-2-methylchromone (noreugenin) 97. Fujita, E. et al[52] isolated noreugenin 97 for the first time from the ethereal extract of the heartwood of Nauclea orientalis(L.)



97 ; R = H

98 ; R = CH_3

Noreugenin 97, $C_{10}H_8O_4$, m.p. 268°C (decomp.), showed[52] the following spectra data. The IR spectrum suggested the presence of hydroxyl groups (3400~2600), an α,β-unsaturated carbonyl group (1660 and 1620cm^{-1} {KBr}). The UV. spectrum

was characteristic of chromone series, giving the absorption maxima at 227, 249, 256 and 295nm . In the NMR spectrum taken in d_6-DMSO, a couple of doublets(J=2Hz) appeared at δ6.32 and 6.20ppm which were assigned to two protons in meta relationship on a benzene ring. The hydroxyl proton signals appeared as singlets at δ10.88 and 12.08ppm. The paramagnetic shift of the latter was due to a hydrogen bond with carbonyl which existed near the hydroxyl group. Another one proton signal on a double bond was observed as a quartet (J = 0.7Hz) at δ6.15ppm while in the NMR spectrum taken in pyridine a doublet (J=0.7Hz) assigned to methyl protons on a double bond appeared at δ2.13.

The structure of noreugenin 97 was proved from the above spectral data and by comparison of the compound and its monoether (got from diazomethane methylation) with the synthetic noreugenin and eugenin 98 respectively.

(b) BIOSYNTHESIS OF CHROMONES.

5,7-dihydroxy-2-methylchromone (noreugenin) 97 has been suggested as a precursor in the biosynthesis of Khellin 99, visnagin 100, visnamminol 101 and hamaudol 102 and a few other related compounds.

Several biosynthetic schemes have been advanced to account for the formation of the above-named compounds, but little experimental evidence has been obtained. Geissman and Hinreiner[53] suggested that the chromones were derived from shikimate and the derivation of some coumarins and chromones from C_5 - substituted phloroglucinol has also been suggested.[54] Egger[55] obtained incorporation of ^{14}C-acetate into Khellol in Eranthis liemalis L. (Ranunculaceae) and showed by chemical degradation that both the benzene and pyrone rings were acetate-derived. He then suggested 5,7-dihydroxy-2-methylchromone 97 as a precursor and speculated that the furan ring was built up from a C_5-unit; peucenin 103 and visnamminol 101 being possible intermediates. Chen, M. et. al[56] showed that ^{14}C-acetate was well-incorporated into khellin and visnagin in cell cultures of Ammi visnaga and showed that the chromone nucleus at least was acetate-derived in these compounds. They also showed that acetate was a precursor of these chromones in the whole plants.

Evidence has been obtained for many of these postulated intermediates between acetate and furanochromones through tracer experiments.[56] The trace experiments provide a number

of facts;

(a) acetate is a good precursor of furanochromones, of visnamminol 101 and of 5,7-dihydroxy-2-methylchromone (noreugenin) 97

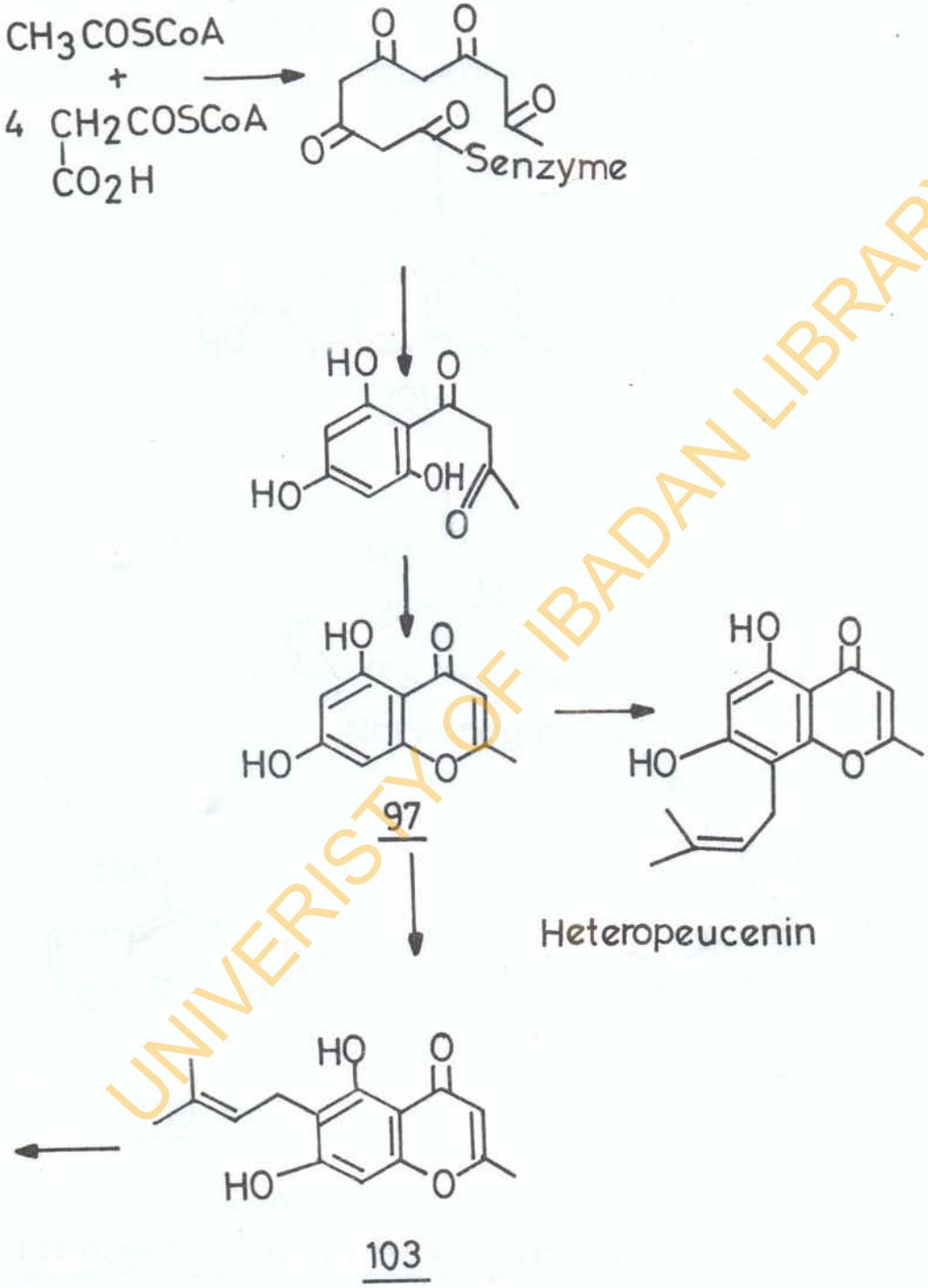
(b) noreugenin is formed naturally in Ammi visnaga;

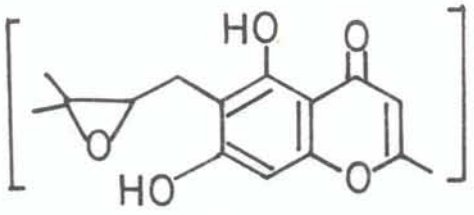
(c) noreugenin specifically metabolized to peucellin 103 visnamminol 101, visnagin 100 and Khellin 99;

(d) Umbelliferone is not a good precursor of any of these compounds.

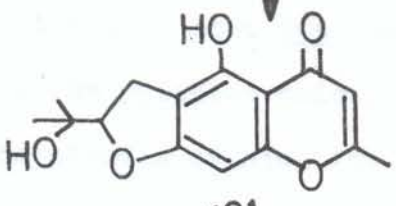
Therefore the biosynthetic scheme 9 was proposed for the constituents of Ammi visnaga. A number of variations in this pathway appear to occur in other chromone bearing plants.

Scheme 9





Peucenin epoxide



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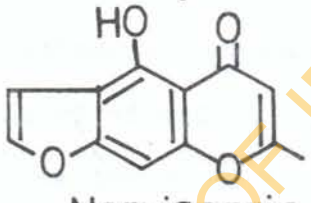
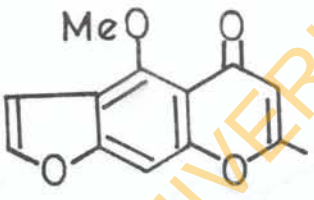


Norvisnagin



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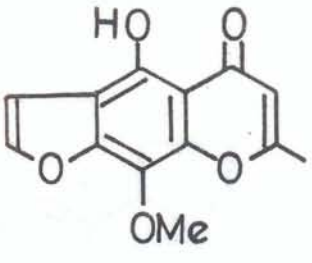
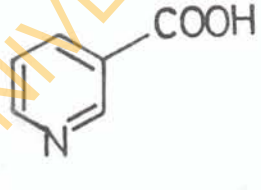


99

Proposed Biosynthetic Pathway for some furano and Other Chromones

IX. BIOSYNTHESIS OF NICOTINE AND NICOTINIC ACID.

For reasons of structural analogy and biological ubiquity, nicotinic acid 104 has been regarded as a possible precusor of the pyridine moiety of the Nicotiana alkaloids.[57] In the biosynthesis of nicotine 105 from nicotinic acid by Nicotiana tobacum, the carboxyl group (C-7) of the nicotinic acid is lost.[58] It is therefore reasonable to assume that the carboxyl group is replaced by the pyrrolidine ring. Indeed, in an electronic mechanism proposed by Dawson[59] for the synthesis of nicotine, and now widely accepted, a 1,6-dihydropyridine intermediate is attacked by an N-methyl-Δ^1-pyrrolidium cation at position (3), with simultaneous decarboxylation.



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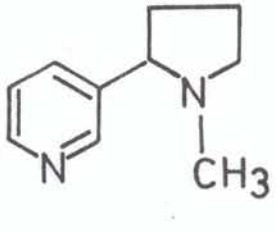
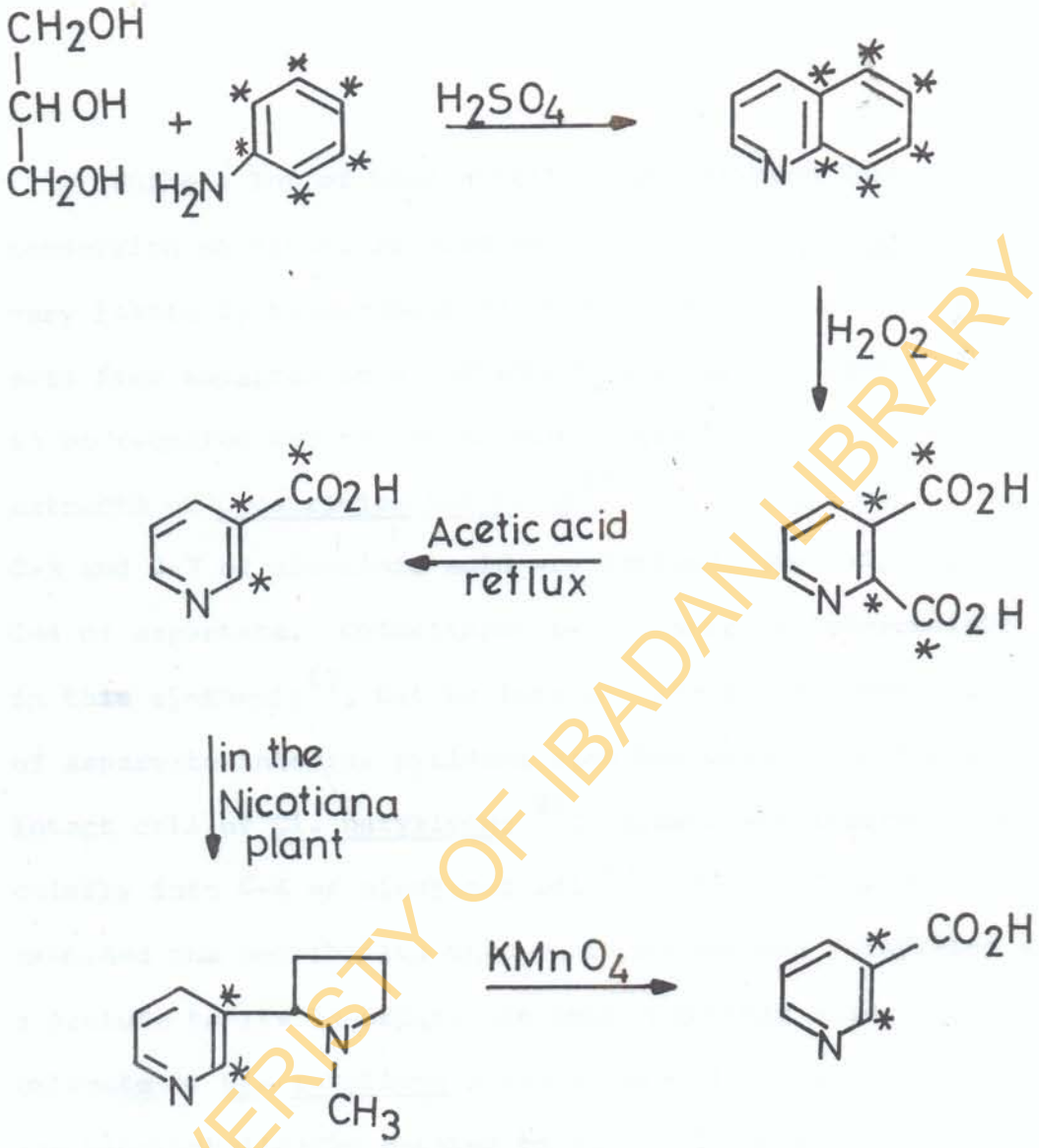


105

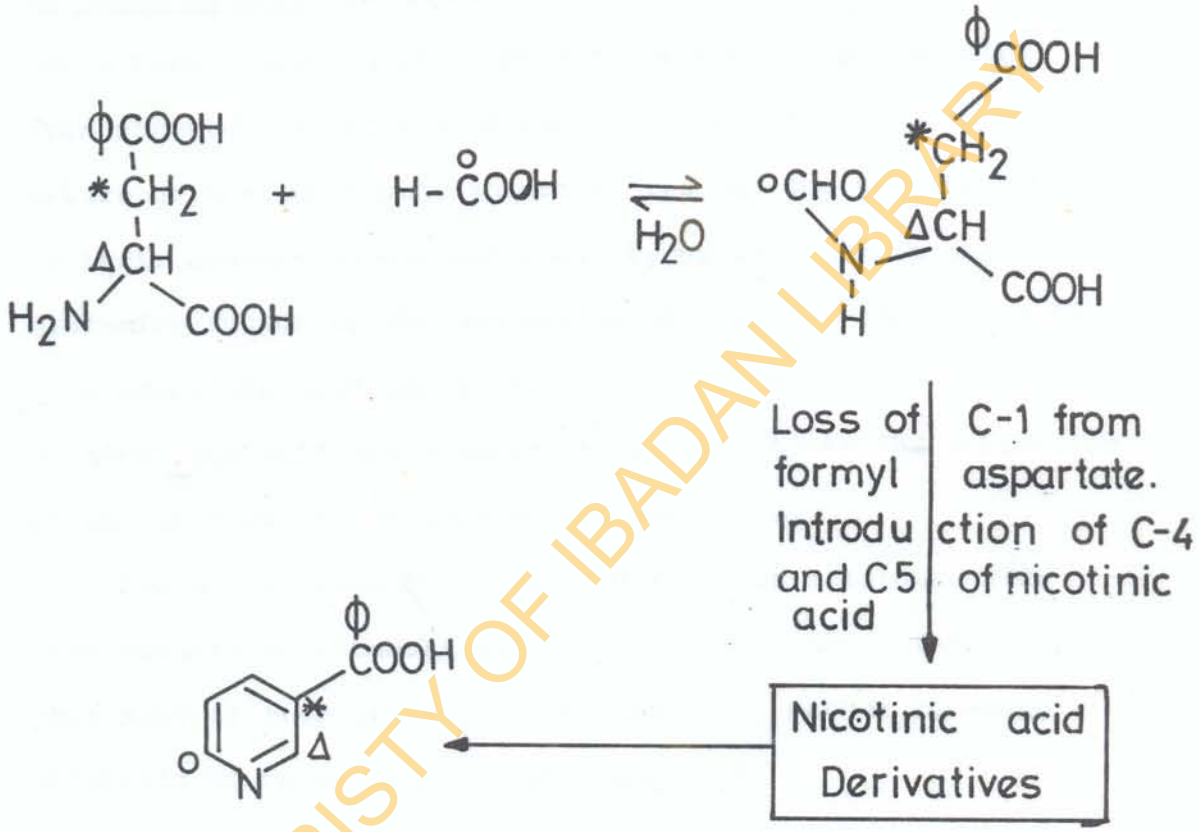
The mechanism also explains the labilization of hydrogen at C-6 of the nicotinic acid, which occurs during the conversion into nicotine.[60] Scott, T.A. et. al[61] while trying to find out whether the attachement of the pyrrolidine moiety occurs exclusively at position (3) of nicotinic acid, synthesized {2,3,7-^{14}C} nicotinic acid according to scheme 10 and administered the acid to the plants of Nicotiana tobacum. The tracer experiments favoured the exclusive attachment of the pyrrolidine moiety at position (3) of nicotinic acid.



Scheme 10

While a lot of biosynthetic work has been done on the conversion of nicotinic acid to nicotine in Nicotiana plants, very little is known about the biosynthesis of nicotinic acid from aspartic acid. Aspartate and formate have been said to be required for the synthesis of nicotinic acid by cell extracts of Clostridium butylicum[62]. In intact cells, C-2, C-3 and C-7 of nicotinic acid are derived from C-2, C-3 and C-4 of aspartate. Quinolinate is probably an intermediate in this synthesis[62], but no intermediate in the conversion of aspartate into the pyridine ring has been identified. In intact cell of Cl. butylicum; ^{14}C-formate was incorporated chiefly into C-6 of nicotinic acid.[63] Scott, T.A. et al.[64] examined the possibility that aspartate becomes formylated as a prelude to its incorporation into nicotinic acid. Cell extracts of Cl. butylicum produce several compounds that are biosynthetically related to nicotinic acid, i.e. NAD, deamido-NAD, nicotinamide, nicotinic acid mononucleotide, nicotinamide-mononucleotide and free nicotinic acid[62]. It was therefore concluded that partially purified Cl.butylicum was able to formylate aspartate to give formyl-L-aspartate, which then acted as an intermediate in the synthesis of

nicotinic acid. This is summarised in Scheme 11.



Φ, Δ, O, * = Specifically labelled C-atoms

Scheme 11

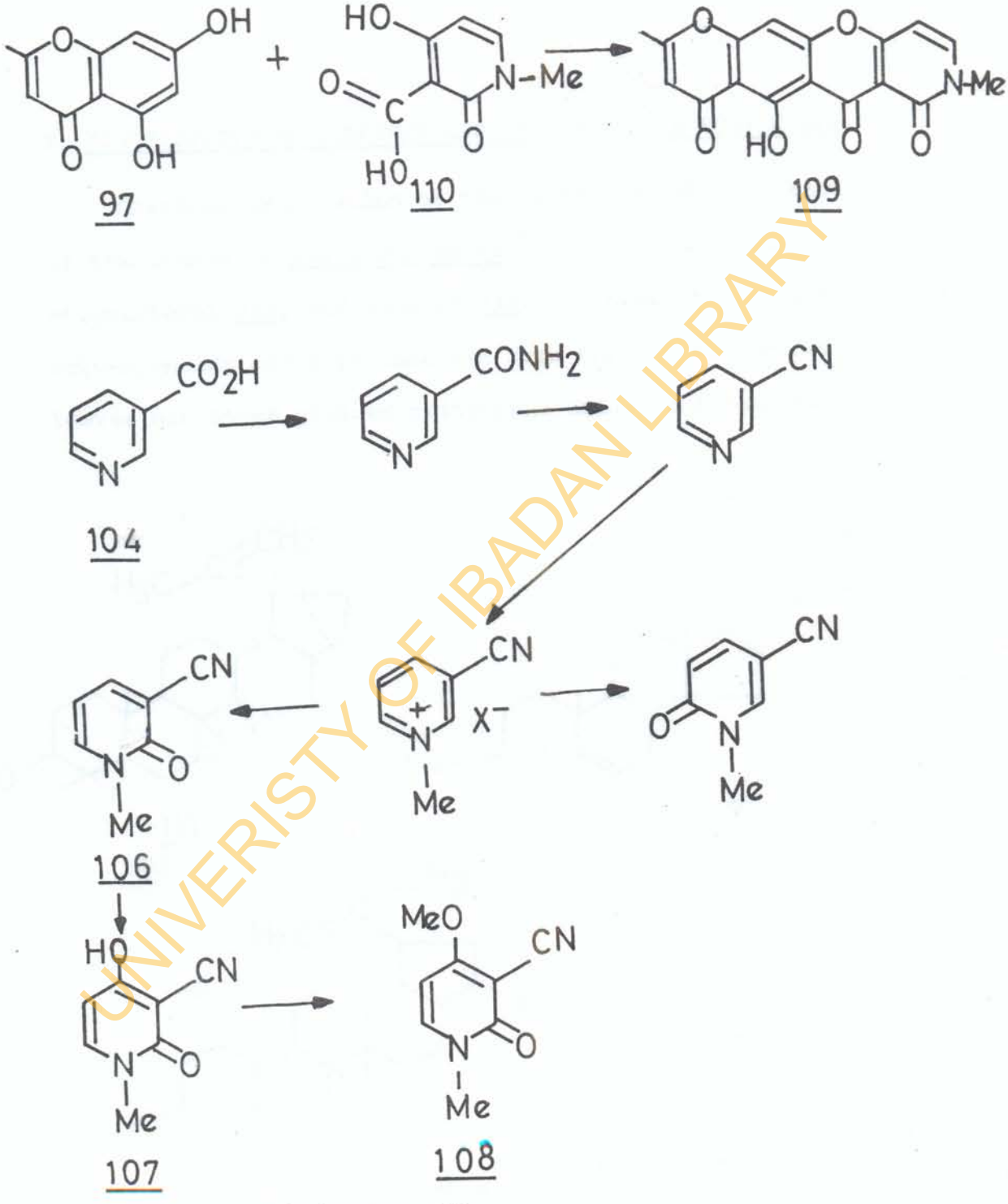
A Schematic representation of the synthesis of nicotinic acid in extracts of Clostridium butylicum

According to Ogasawara et al[64] extracts of Escherichia coli synthesized nicotinic acid from aspartate and a three carbon unit. The synthesis of nicotinic acid from formyl-L-aspartic acid must involve several stages, the nature of the subsequent intermediates and the enzymes concerned in their conversion was unknown. Pyruvate, acetate and glutamine supported the conversion of formyl-L-aspartic acid into nicotinic acid and although the origins of C-5 and C-4 of nicotinic acid are unknown, they must lie in the metabolism of one or more of the above-named compounds.

For a long time it was considered that alkaloids were end-products of metabolism. However, it is now being realised that many of them are rapidly metabolized, either to other alkaloids or to non-alkaloidal compounds.[65]

The specific intermediates between nicotinic acid 104 and ricinine 108 are unknown, a reasonable proposed biosynthetic pathway[65] is illustrated in scheme 12.

In the biosynthesis of an alkaloid 109, a reasonable speculation could be that there was a condensation between 5,7-dihydroxy-2-methylchromone 97 and the acid 110 formed from 3-cyano-4-hydroxypyridine 107.

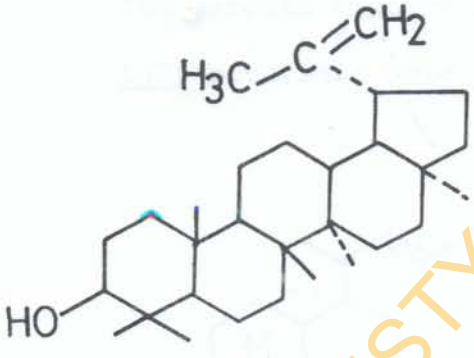


Scheme 12

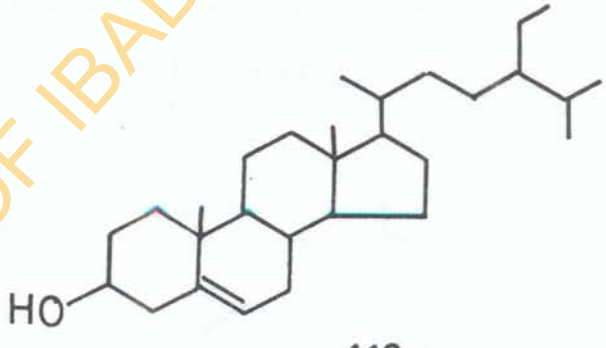
Proposed enzymatic reactions in Ricinus communis

X. DISTRIBUTION OF TERPENES AND TERPENOIDS IN RUBIACEAE.

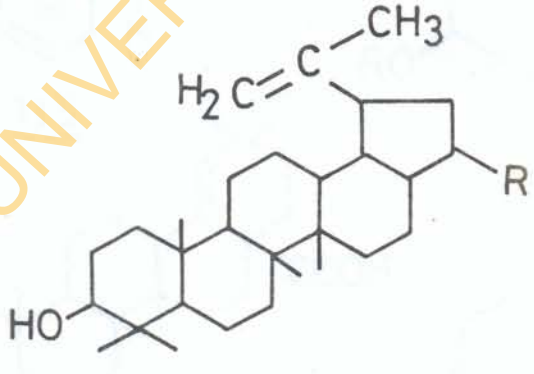
Chemical examination of the petroleum ether extract of the leaves of Ixora chinensis[66] gave lupeol 111, stigmasterol 112, and betulin 113. Neither sterols nor triterpenoids could be isolated from the stems, and neither leaves nor stems yielded triterpene acids or saponins.



111



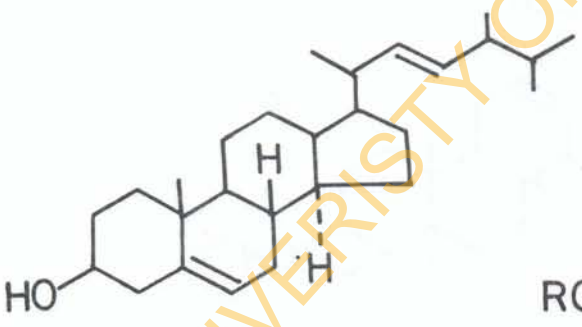
112



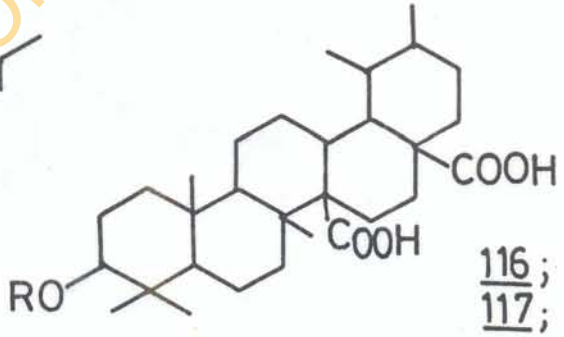
113 ; R = CH_2OH

114 ; R = COOH

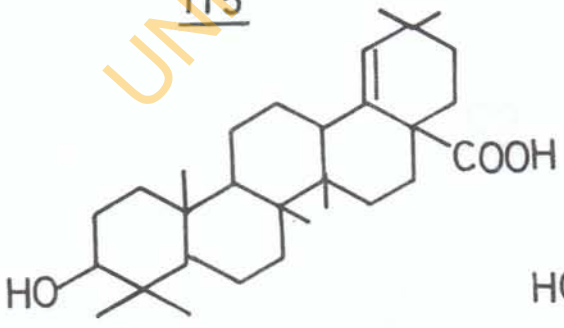
The petroleum ether extracts of the leaves of Adina pilulifera[67] yielded β-sitosterol 115, stigmasterol 112 and a mixture of saponins, which gave quinovic acid 116 and its acetate 117 on hydrolysis. This is the first report of the natural occurence of the acetate of quinonic acid, which was isolated through its dimethyl ester. The constituents of the petroleum ether extracts of the stems were identical with those of the leaves[67] but in addition, the saponin mixture from the stems yielded morolic acid 118, betulinic acid 114 and cincholic acid 119.



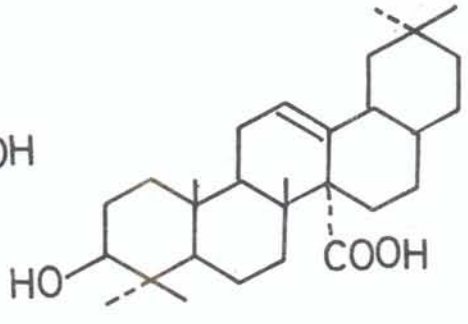
115



116; R = H
117; R = AC

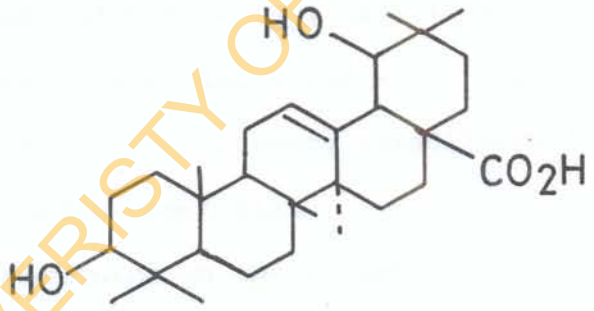


118



119

The whole plant of Randia spinosa (Thumb Poir) known also as mountain pomegranate, was investigated[68] as it has sharp thorns and small leaves which were not easily separable from the stems. From the light petroleum extract, β-sitosterol, and stigmasterol were isolated. Further extraction with ethanol gave a saponin mixture, which on hydrolysis yielded oleanolic acid, siaresinolic acids, and spinosic acid A 120.



120

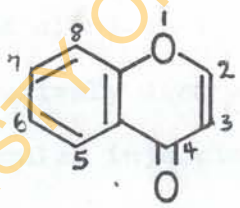
XI. COMPARISON OF BRONCHIODILATOR ACTIVITIES OF CHROMONES WITH THAT OF KHELLIN

Khellin 99 which a number of pharmaceutical laboratories in Egypt and in the United States prepare on a commercial scale and generally dispense in the form of tablets or injectable solutions forms one of the active principles of Khallah plant (Ammi visnaga L.)[69]. It is known to the Egyptians to be useful in relieving the pain of renal colic and ureteral spasms and often to facilitate the passing of ureteral stones. Following the elucidation of the chemical structure of Khellin 99, additional pharmacological properties were discovered which led to its chemical application as a coronary vasodilator in bronchial asthma and in angina pectoris.

Consequent on the report of the capability of khellin to effectively relax the bronchi[70], Willey, P.F.[71] thought that more readily accessible chromones having bronchiodilator activity equal to or greater than that of khellin would be desirable. This was because of the complexity of khellin 99 and difficulty in synthesizing or isolating it from natural sources.

The tests for bronchiodilator activity were run on an isolated guinea pig tracheal chain. The activity of the compound being tested was compared to the activity of epinephrine. Results are expressed (Table 2) in the number of micrograms of epinephrine required to equal the activity of one milligram of the compound tested. Very little correlation of structure with activity was observed. Those chromones having no 2-methyl substituent showed negligible activity. The activity of khellin 99 on the same test is also shown for the purposes of comparison.

TABLE 2.



121

Number	Substituents	Activity γ -epinephrine/mg.
1.	6-methoxy	0
2.	7-methoxy	0.6
3.	2-methyl-6-methoxy	4
4.	2-methyl-7-methoxy	5
5.	2-Bromomethyl-7-methoxy	1
6.	2-methyl-5,7-dimethoxy	<1
7.	2-methyl-7,8-dimethoxy	3
8.	2-methyl-5,7,8-trimethoxy	<3
	Khellin	30

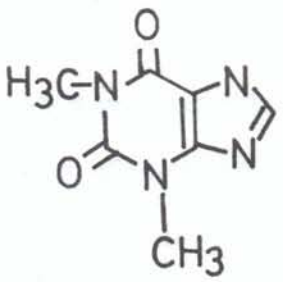
XII PHARMACOLOGICAL ACTIVITIES OF SOME SELECTED ALKALOIDS OF RUBIACEAE

Quinine 56 preparations have been known and used for centuries in the treatment of malaria[43]. The specific action of emetine 48 against pathogenic amoeba in human amoebic dysentry makes it a valuable drug. It was introduced by Rogers. L.[38] in 1912 for this purpose, and afterwards in the treatment of amoebic hepatitis. After a comparative investigation of the Ipecac alkaloids and some of their derivatives, Dale and Dobell[38] concluded that emetine 48 acts through the host rather than on the parasite, that is, changes in the blood render it amoebicidal. Satisfactory cultural methods in vitro, which permit pharmacological investigations[72-73] and clinical tests have been devised. Emetine is almost exclusively used as hydrochloride for hypodermic or intramuscular injections.[38]

There is one example of animal poisoning which requires special mention - the doping or illicit medication of racing animals. By definition, doping is the administration of a drug to an animal in order to affect its speed, stamina, courage, or conduct in a race.[74] Horses are the animals

most frequently subjected to this practice, but greyhounds, racing pigeons and (possibly) bulls are sometimes doped, while athletes may dope themselves. Doping usually consists either of the administration of a stimulant to make an animal go faster (doping to win) or of a sedative to make it go more slowly (doping to lose or "nobbling"), but other procedures such as the use of local anaesthetics to mask lameness, of tranquilizers to control a highly spirited animal and of sex hormones for a female in estrus are also employed. It is as stimulants that alkaloids most frequently find employment. Possibly, caffeine 122 has been used more frequently than any other drug. It is cheap, easy to obtain, and reasonably effective. A horse is more alert, gets away to a better start and responds more quickly to its rider. Strychnine has also been used extensively for this purpose, but its actions seem less reliable. Both morphine and heroin, which act as stimulants in the horse, have also been widely used in the past. If the dose and the timing are both correct a horse doped with morphine will run far above its normal form. As these substances are alkaloids, the idea has arisen that any

alkaloid will do, and so much unlikely compounds as atropine, ephedrine, yohimbine 32 and quinine 56 have been employed. Cocaine has also been used with limited success. The modern tendency however is to use synthetic drugs such as amphetamine.



122

Alkaloids are not used as sedative drugs in the horse; barbiturates or chloral are usually employed but codeine and quinine 56, the latter in contradistinction to its use as a potential stimulant in the horse, have been used for this purpose in greyhounds, which are nearly always doped to lose. Racing pigeons are sometimes given amphetamine to delay the onset of fatigue. Bulls are reported to have been quitened with tranquilizers. The doping of athletes consists of self-medication with drugs of amphetamine type. There appear to be no fatal cases

of poisoning in man by caffeine 122 on record[74], but doses over 1gm. may produce alarming symptons, including tachycardia and sensory disturbances.

Objectives of this work on Schumanniophyton magnificum

The use of S. magnificum in herbal medicine and especially its use in the treatment of snake-bite stimulated our interest in the chemical investigation of the plant. There are still a limited number of serums which do not keep for very long, that are used in the treatment of snake bites.

Therefore the isolation, characterisation and establishment of the biological activities of the extractives from the plant could add to the number of the curatives for snake-bites and other diseases. Apart from these objectives the chemotaxonomy of plants and the chemistry of complex secondary metabolites from plants continue to be of interest.

In the course of this work, results of the chemical investigation of Schumanniophyton problematicum was reported[6]. The following result and discussion is based on the investigation of S. magnificum.

RESULTS AND DISCUSSION

The specimens of Schumanniophyton magnificum (Harms) used in this investigation were collected at Sapoba Forest Reserve, in September, 1976 in Bendel state of Nigeria. They were authenticated at source and confirmed to be S. magnificum (Harms) at the Federal Department of Forest Research, Ibadan, where a herbarium specimen is kept. The root-bark of Schum. magnif. was extracted with hexane and methanol respectively. The components of hexane extract (about three major compounds) which happened to be non-alkaloids were not of any interest to us. The methanol extract was found to contain alkaloids which were of great interest to us, hence the work was based on the methanol extract.

The methanol extract of the root-bark of S. magnificum was concentrated (by evaporating the methanol). This yielded a precipitate with an oily upper layer. The oily upper layer was decanted leaving the precipitate behind. Both the precipitate and the oily layer were extracted separately with chloroform. The chloroform extracts of the precipitate and the oily portion contained essentially the same components. The residue left behind after extracting the oily layer with chloroform several times was a water-soluble oil which probably contained glycosides. The compounds of the above chloroform extracts were very polar, so there was no good separation on the t.l.c. plate developed in a mixture of benzene and ethyl acetate (whatever the ratio) but a mixture of chloroform and ethyl acetate (3:1) was suitable for the development on the t.l.c. plate, giving four spots.

Evaporation of the chloroform extracts afforded a crude solid.

The components of this crude solid were separated on a column of silica gel. The first compound designated SRB_1 (R_f = 0.50) and the most polar compound on the t.l.c. plate, SRB_4 (R_f = 0.10) were obtained pure from the column chromatography. Two other compounds, SRB_3 and SRB_3' came down from the column as a mixture. Pure SRB_3 (R_f = 0.18) was obtained from the fractional crystallization of a mixture of SRB_3 and SRB_3' in methanol. Recrystallization of the residue left after the fractional crystallization gave crystals of SRB_3'. A mixture of SRB_2 (R_f = 0.30) and SRB_3'' (R_f = 0.18) was eluted from the column, which was only separated by treating the mixture of the two compounds with aqueous ammonia. SRB_2 went into the aqueous ammonia, while SRB_3'' was left behind. SRB_3'' was recovered by filtration. The filtrate which was the aqueous ammonia portion was acidified. This gave the precipitate of SRB_2 which was dried by filtration.

Apart from SRB_1 and SRB_3', all the four others gave positive alkaloid test with Dragendorff's reagent. SRB_3' was later proved to be a nitrogen-containing compound (microanalysis). An attempt made at separating the alkaloids from SRB_1 (non-alkaloid) by treatment with an acid to form salts of the alkaloids proved unsuccessful. There was no separation also when the crude solid was treated

with a base. This was aimed at forming the salts of the phenolic compounds, so that the non-acidic compounds could be extracted. Since all the compounds went into the base layer, it suggested that the alkaloids were phenolic.

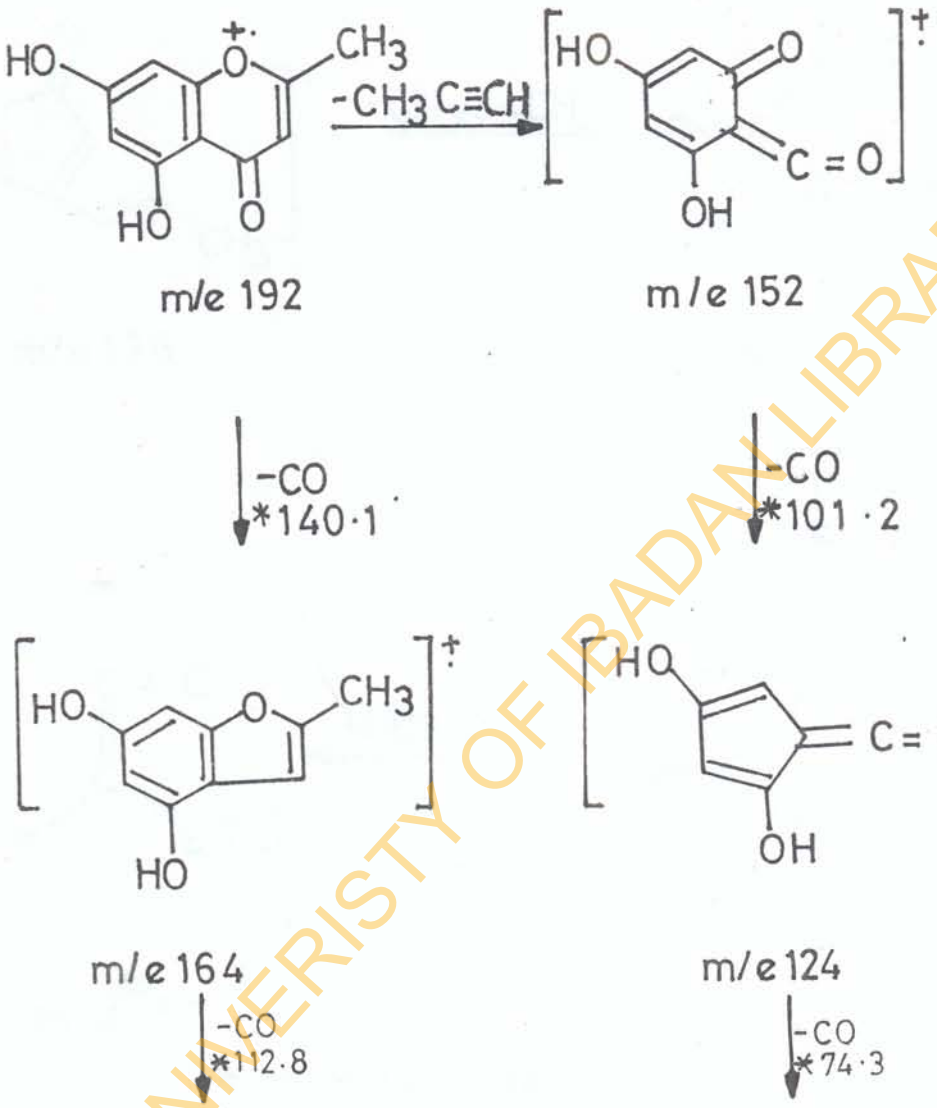
Determination of the constitutional formula of SRB_1

The compound SRB_1 which was proved to be a chromone, had a m.p. 274-276°, when recrystallized from ethanol but recrystallization from a mixture of $MeOH/CHCl_3$ afforded some needle-like crystals, m.p. 265-266° (decomp.).

It's mass spectrum indicated a parent peak at m/e 192 which was also the base peak (low resolution mass spectrum) and the molecular ion, M^+ 192 (70%) remained the same in another mass spectrum (high resolution mass spectrum), but the base peak was at m/e 69. In both cases, the molecular ion was in agreement with the molecular formula, $C_{10}H_8O_4$ (microanalysis). The following important peaks M^+ 192 (70%), 164 (96%), 163 (52%) 152 (26%), 136 (18%), 124 (64%) 96 (39%), 95 (22.5%) and 69 (100% - base peak), are in accordance with the well established[77] fragmentation pattern for chromones.

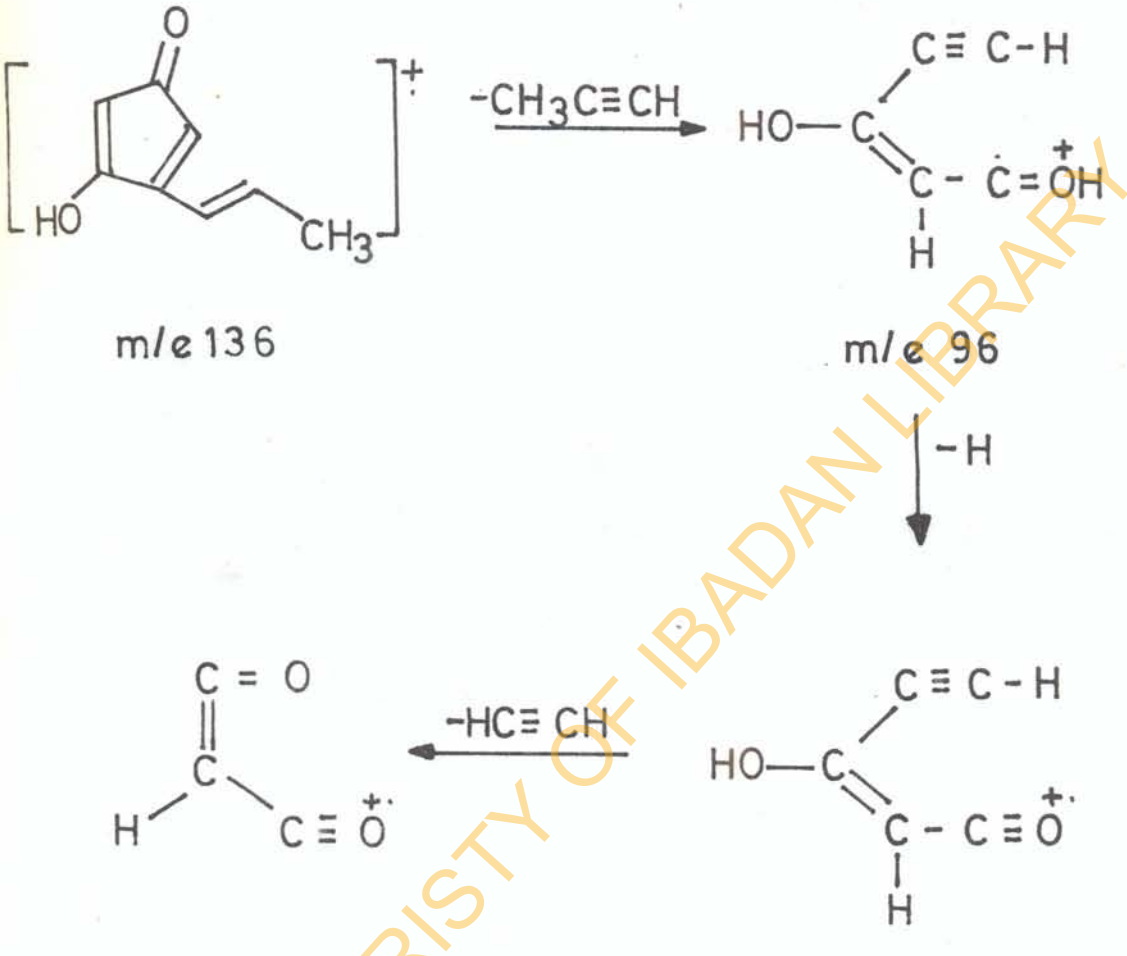
The mass spectra of a number of natural chromones and their derivatives indicated that a characteristic fragmentation resulted by collapse of the γ-pyrone system in a retro

Diels-Alder type of reaction[77]. Other notable features included the expected multiple loss of carbon monoxide, loss of methyl radical from methoxylated products and sometimes the loss of a hydrogen atom, water and 29 mass units (CHO). Substitution of the benzenoid ring with hydroxyl groups does not alter the general fragmentation pattern. The fragmentation pattern of 5,7-dihydroxy-2-methylchromone 97 shown in scheme 13 is based on the already established pattern[77] and the metastable peaks obtained from the mass spectrum of 97.



Scheme 13

Scheme 13 contd.



* – Metastable peak

Proposed fragmentation pattern of 5,7-dihydroxy-2-methylchromone.

Fig. 1

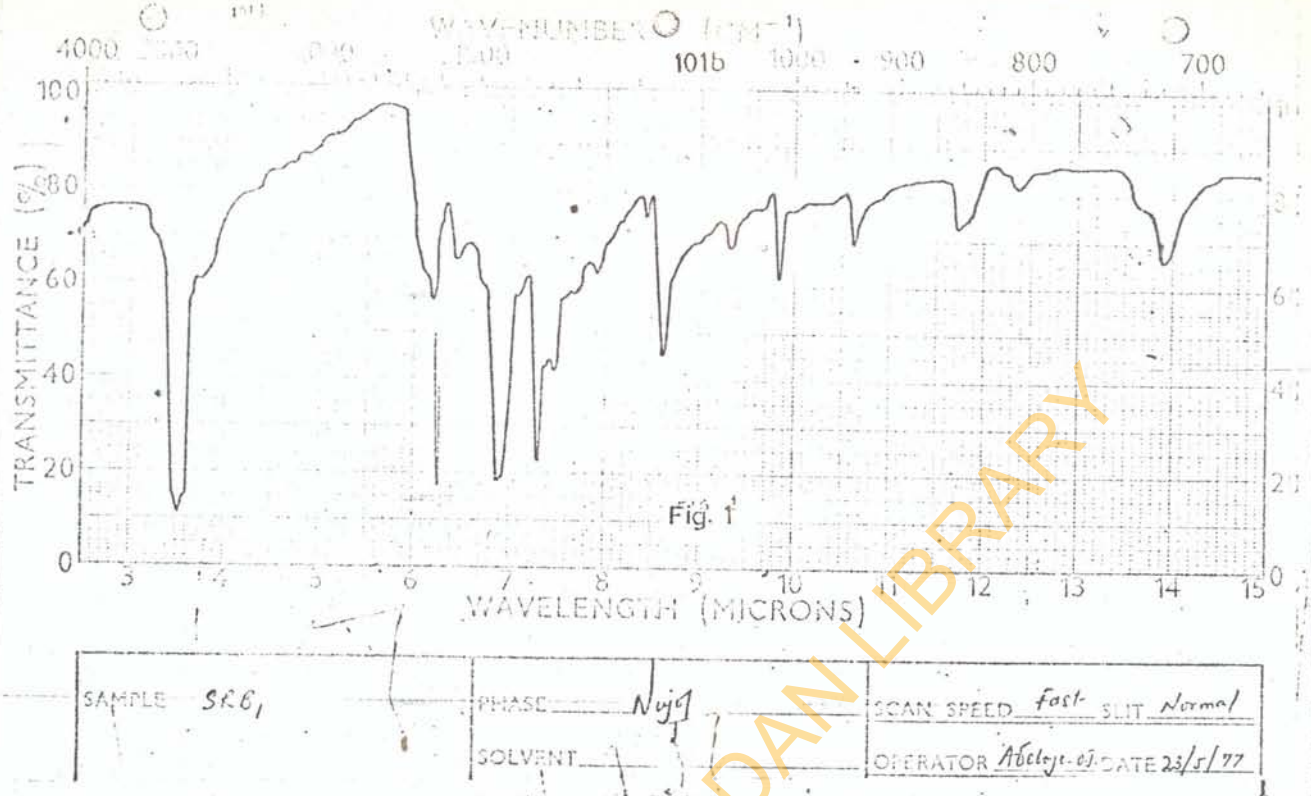


Fig. 1

SAMPLE SRC1 PHASE Nujol SCAN SPEED Fast SLIT Normal

SOLVENT OPERATOR Abeloje-oj DATE 23/5/77

101b

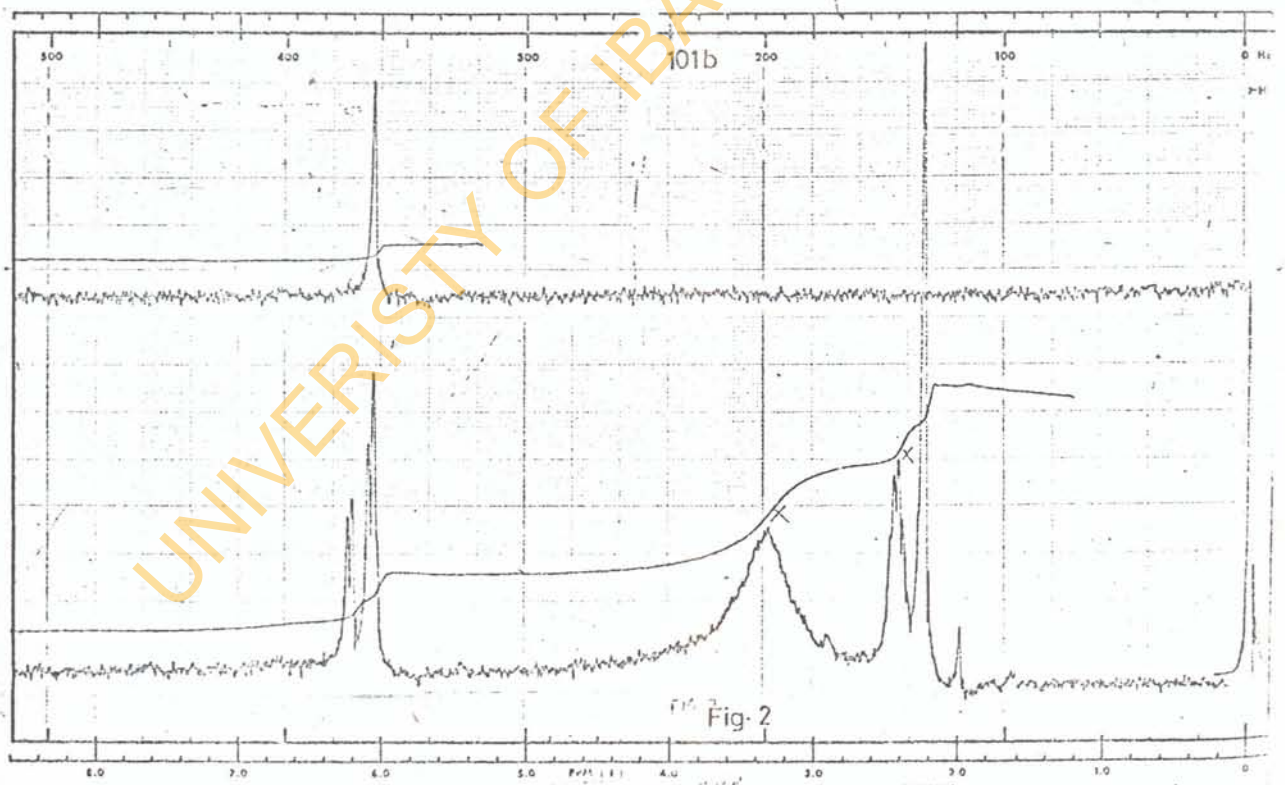


Fig. 2

8.0 7.0 6.0 5.0 PPM (δ) 4.0 3.0 2.0 1.0 0

MANUAL AUTO SAMPLE: SRC1 REMARKS:

SWEEP OFFSET (Hz): 600 SWEEP TIME (SEC): (350)

SPECTRUM AMPLITUDE: 5 SWEEP WIDTH (Hz): (500)

INTEGRAL AMPLITUDE: 5.0 FILTER: (2)

SPINNING RATE (RPS): 0 RF POWER LEVEL: (.05) SOLVENT: CHCl3

X – absorption by solvent

DATE: 24/5/77 OPERATOR: J. O. Abeloje 60 MHz NMR SPECTRUM NO.

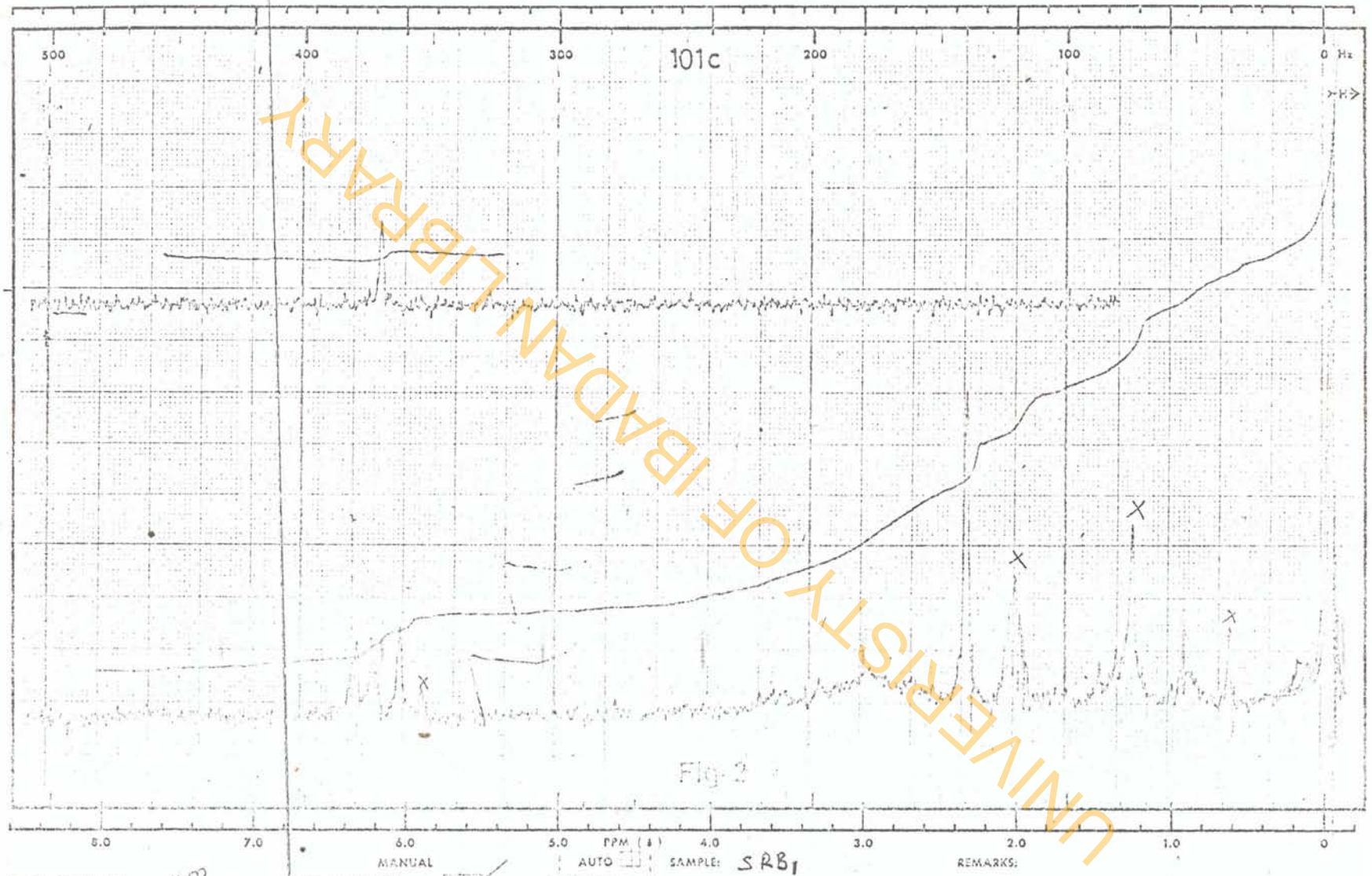


Fig 2

SWEEP OFFSET (Hz): 1500
SPECTRUM AMPLITUDE: 40
INTEGRAL AMPLITUDE: 10.9
SPINNING RATE (RPS): 0.32

MANUAL ☐ AUTO ☐

SWEEP TIME (SEC): 250
SWEEP WIDTH (Hz): 500
FILTER: 2
RF POWER LEVEL: 0.05 (.05)

SAMPLE: SRB1

SOLVENT: CD_3COCD_3

REMARKS:

x – absorption by Solvent

DATE: 6/2/78

OPERATOR: O. Adeboye

60 MHz NMR
SPECTRUM NO.

The IR spectrum (FIG 1) suggested the presence of hydroxyl groups (3420–2620cm^{-1}), though only one phenolic hydroxyl group was observed in the NMR spectrum taken in d_6-DMSO and this might be due to proton exchange of the non-hydrogen bonded hydroxyl group with the deuterium of the solvent. An α,β-unsaturated carbonyl group characteristic of the chromone series appeared at 1660cm^{-1} and 1620cm^{-1} (KBr), 1560 and 1500cm^{-1} (>C=C< of benzene ring), 1165cm^{-1} (ether linkage), 890, 845 and 820cm^{-1} (substituted aromatic ring).

SRB_1 gave a positive ferric chloride test which indicated that it was phenolic. From the NMR spectrum (FIG.2) of SRB_1 taken in d_6-DMSO (SRB_1 not soluble in $CDCl_3$), a pair of doublets (J = 2Hz) appeared at δ6.18 and δ6.28 which were assigned to two meta-coupled protons on a benzene ring. A three proton singlet assigned to a methyl group at 2-position of the γ-pyrone appeared at δ2.27 and one proton signal was observed as a broad singlet at δ6.08, which was assigned to a proton at 3-position of γ-pyrone. The only phenolic proton signal observed appeared at δ12.7. The paramagnetic shift was due to a hydrogen-bonding with the neighbouring carbonyl group.

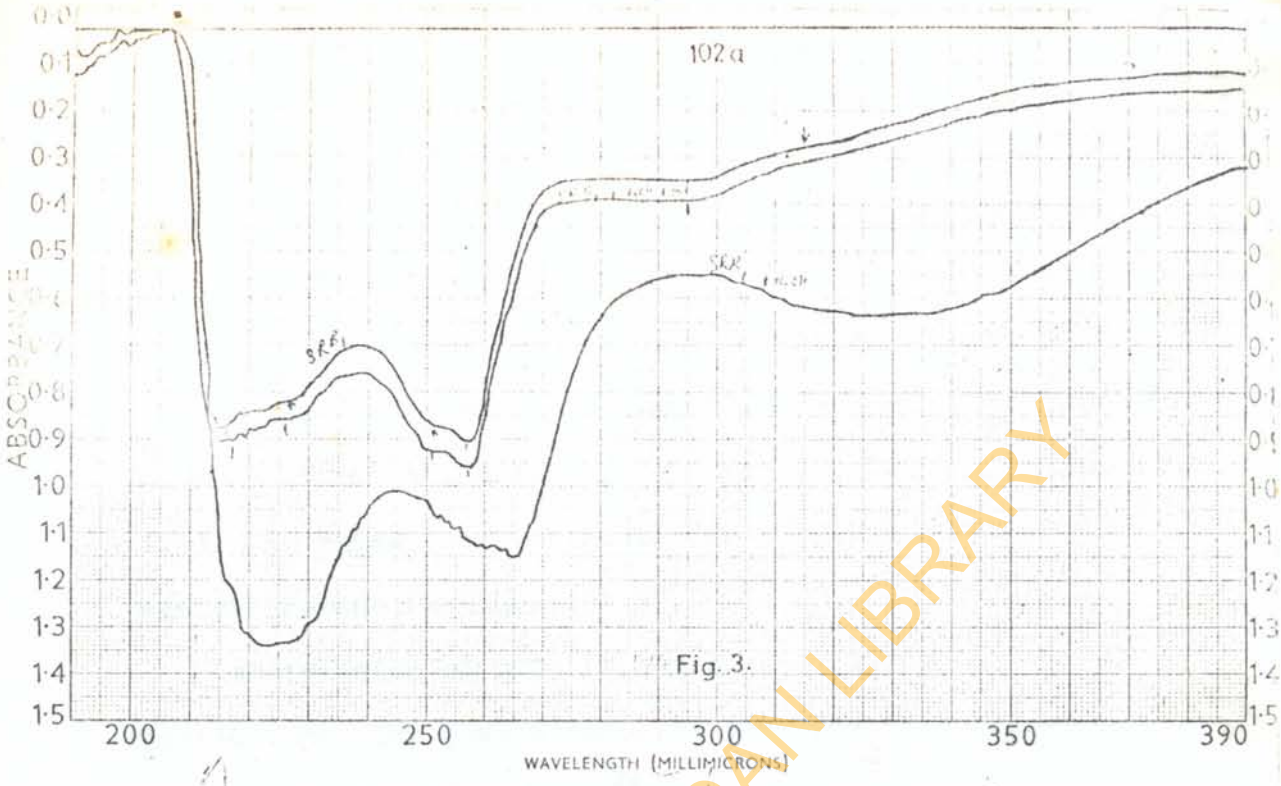


Fig. 3.

SAMPLE SRB1	CURVE NO.	SCAN SPEED Fast	OPERATOR I. Adeboye
	CONC. 4 mg/100cm³	SLIT Normal	DATE 18/5/77
ORIGIN	CELL PATH	REMARKS	
SOLVENT MeOH	REFERENCE MeOH		

PART NO. 202-1511 PERKIN-ELMER LIMITED

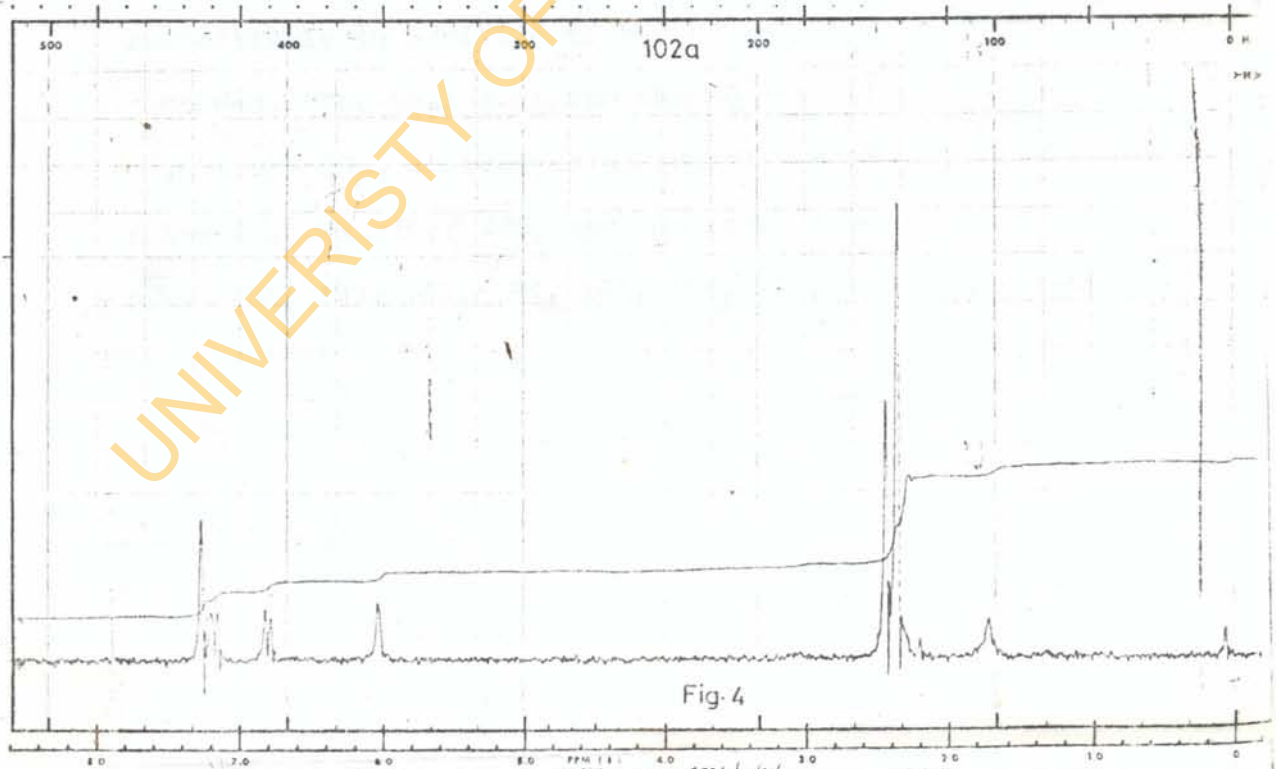


Fig. 4

MANUAL ✓ AUTO ☐ SAMPLE: Acetylated SRB1 REMARKS: No peaks after 8.0 ppm

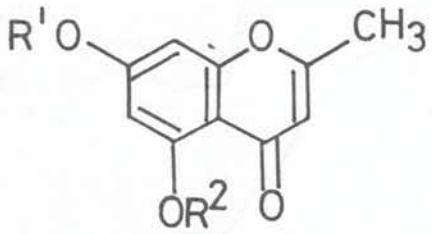
SWEEP OFFSET (H): SWEEP TIME (SEC): (350)
SPECTRUM AMPLITUDE: 2.0 SWEEP WIDTH (Hz): (500)
INTEGRAL AMPLITUDE: 5 FILTER: (3)
SPINNING RATE (CPS): ~40 RF POWER LEVEL: 0 (.05) SOLVENT: CDCl3

13/5/77 60 MHz NMR

The ultraviolet spectrum (FIG.3) was characteristic of the chromone series, giving the absorption maxima at 217 (log ϵ = 4.21), 227 (log ϵ = 4.18), 251 (log ϵ = 4.21), 257 (log ϵ = 4.22), 295 (log ϵ = 3.83) and 325nm (log ϵ = 3.68). The literature[52] reported the following absorption maxima: 227 (log ϵ = 4.08), 249 (log ϵ = 4.13) 256 (log ϵ = 4.13) and 295nm (log ϵ = 3.65) for 5,7-dihydroxy-2-methylchromone (noreugenin) 97.

Acetylation of SRB_1 in a mixture of pyridine and acetic anhydride gave a colourless crystalline diacetate 123, m.p. 137-139° (lit.[75], 139.5 - 140.5°)

The NMR spectrum (FIG 4) of the diacetate of SRB_1 showed two enol acetate signals at δ2.30 and δ2.40 respectively in addition to the proton signals of the parent compound. The mass spectrum showed the molecular ion, M^+, at m/e 276, indicating an addition of 84 units to the molecular weight of SRB_1 and had other fragment ions at m/e. 234, 193, 192, 164, 163, 151, 124, 123, 96, 69 and 43.

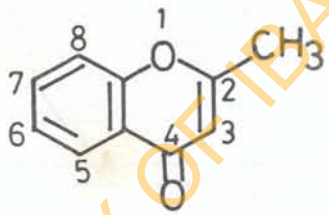


123 ; $R^1 = R^2 = Ac$

124 ; $R^1 = CH_3$; $R^2 = H$

125 ; $R^1 = CH_3$; $R^2 = Ac$

126 ; $R^1 = R^2 = CH_3$



127

Diazomethane methylation of SRB_1 gave one single compound (t.l.c). This was confirmed to be the monomethylether from the spectral properties. The monomethylation product pointed to the fact that one of the hydroxyl groups was involved in hydrogen-bonding, while the free one was methylated. The monomethylether had a m.p. 116-117° (literature[75], m.p. 119-120°) and analysed for $C_{11}H_{10}O_4$. It was identical in mixed mp and spectral properties with eugenin 124 that is, monomethylether of 5,7-dihydroxy-2-methylchromone.

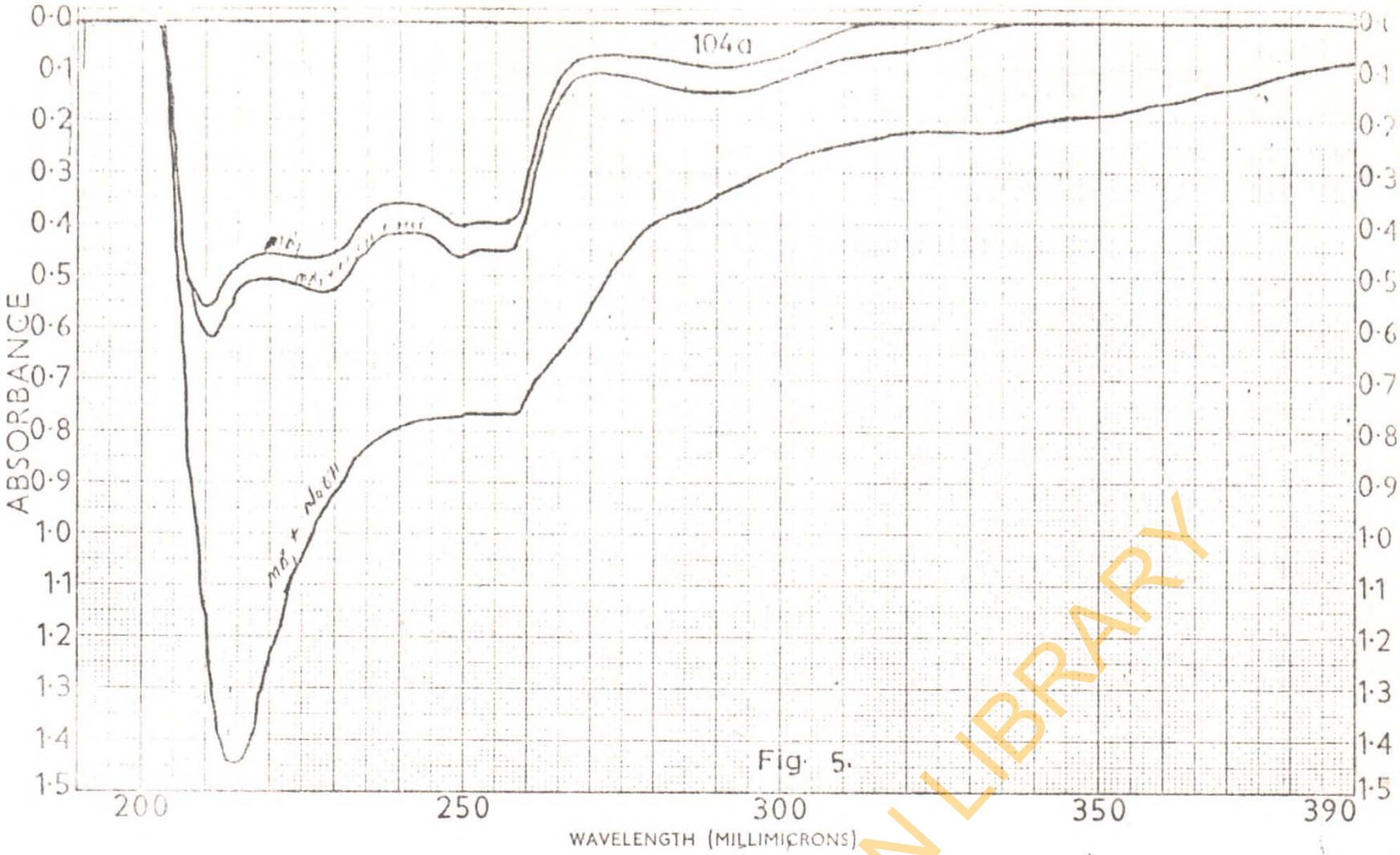


Fig. 5.

SAMPLE MG	CURVE NO.	SCAN SPEED Slow	OPERATOR AC60yc
	CONC. 4mg/1000cm³	SLIT Normal	DATE 12/5/77
ORIGIN	CELL PATH	REMARKS	
SOLVENT MeOH	REFERENCE MeOH		

PART NO. 202-1511

PERKIN-ELMER LIMITED

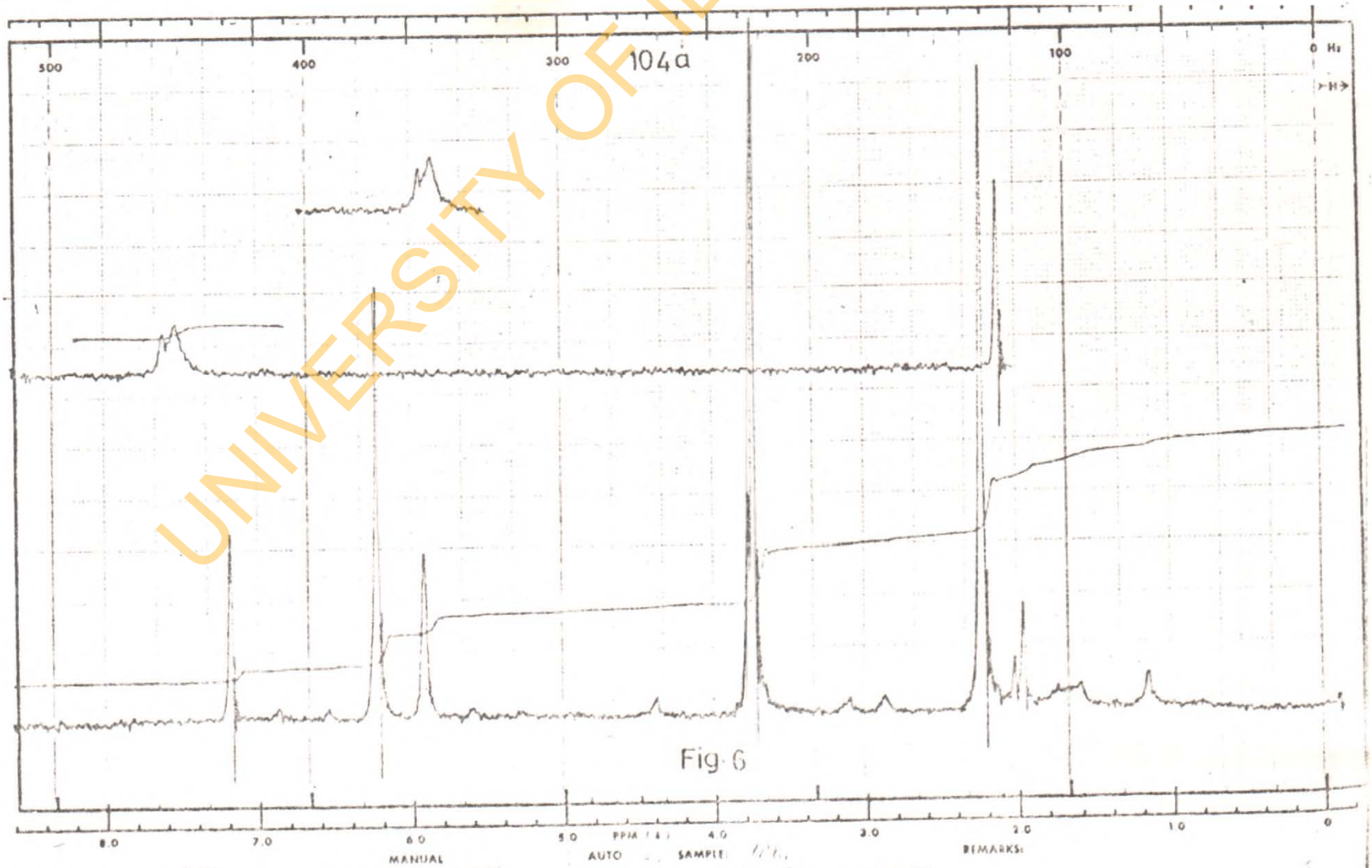


Fig. 6

MANUAL AUTO

SWEEP OFFSET (Hz): 250
SPECTRUM AMPLITUDE:
INTEGRAL AMPLITUDE:
SPINNING RATE (RPS):

SWEEP TIME (SEC): (250)
SWEEP WIDTH (Hz): (500)
FILTER: (2)
RF POWER LEVEL: (.05)

SAMPLE:
SOLVENT:

REMARKS:

DATE: 20/10/77 OPERATOR:

60 MHz NMR
SPECTRUM NO.

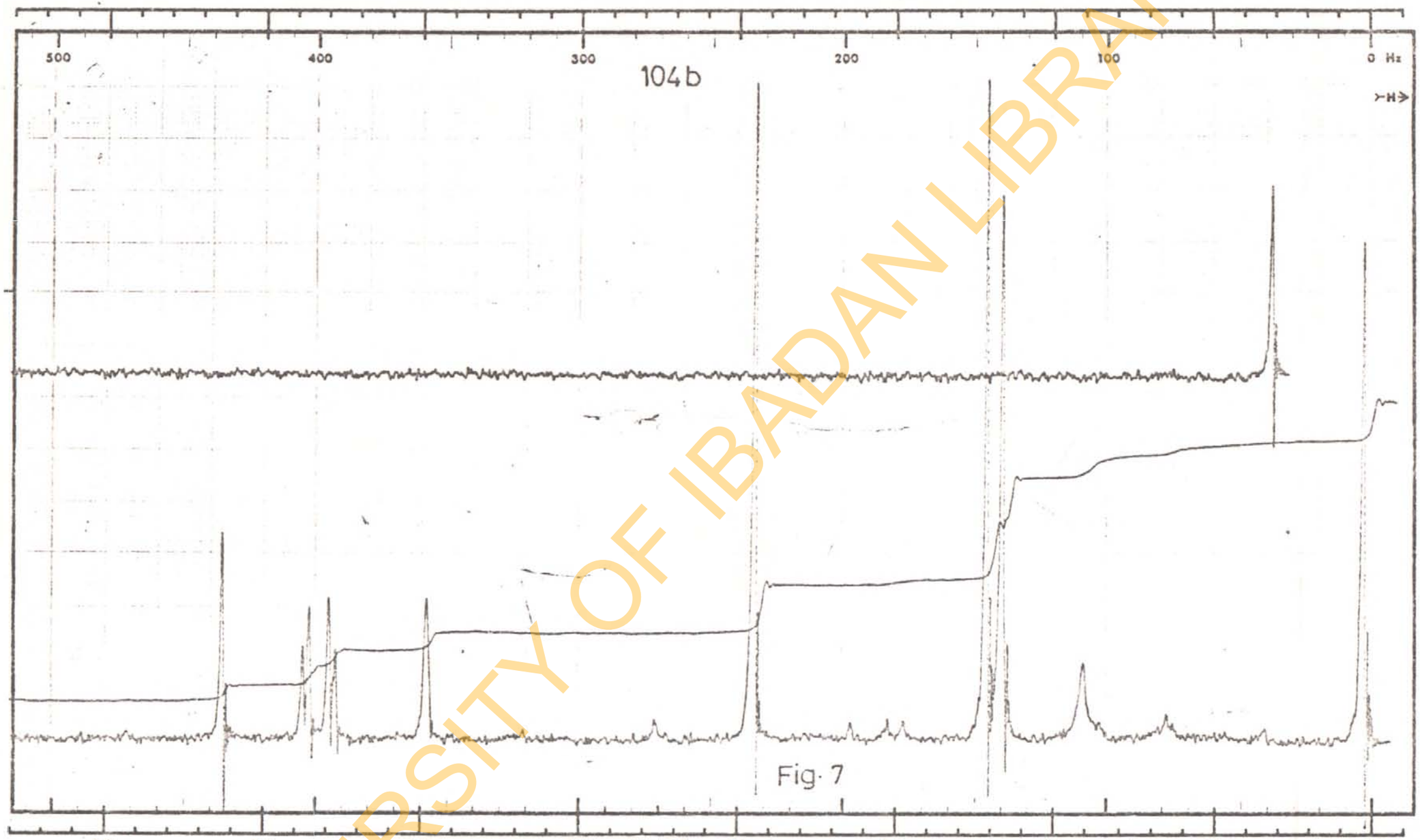


Fig. 7

SWEEP OFFSET (Hz): 400
SPECTRUM AMPLITUDE: 25
INTEGRAL AMPLITUDE: 4.9
SPINNING RATE (RPS): 29

MANUAL
SWEEP TIME (SEC): 80 250
SWEEP WIDTH (Hz): 25 50 100 250 500
FILTER: 1 2 3 4 5 6 7 8
RF POWER LEVEL: 0.03

AUTO ☐
(250)
(500)
(2)
(.05)

SAMPLE: Acetylated Methyl

REMARKS: No peaks offset.

SOLVENT: $CDCl_3$

DATE: 11/8/77

OPERATOR: B. O. Adelaye

60 MHz NMR
SPECTRUM NO.

The ultraviolet spectrum (FIG. 5) of the monomethyl-ether of SRB_1 showed absorption maxima λ_{max} (nm) at 210 (log ϵ = 4.43), 230 (log ϵ = 4.34), 250 (log ϵ = 4.29), 257 (log ϵ = 4.28) and 295 (log ϵ = 3.7). These compared fairly with the ultraviolet spectrum of eugenin 124, which showed[44,78] absorption maxima λ_{max} (nm) at 248 (log ϵ = 4.3), 257 (log ϵ = 4.3), 288 (log ϵ = 3.9) and 318 (log ϵ = 3.7). The NMR spectrum (FIG. 6) with one methoxyl proton signal at δ3.8 (3H, s) and the phenolic hydroxyl signal at δ12.7 (1H, s, which disappeared with D_2O), confirmed the monomethylation. Comparison of the mass spectrum of the monomethylether of SRB_1 with that of eugenin[77] further established the identity.

The successful acetylation of the monomethylether 124 of SRB_1 to give a compound identical with the acetate 125 of eugenin, served to prove that there were two phenolic hydroxyl groups. The acetate 125 had a m.p. 150-151° (literature[75] 152.5 - 153.5°). The NMR spectrum showed the following proton signals δ2.30 (3H, s, methyl)

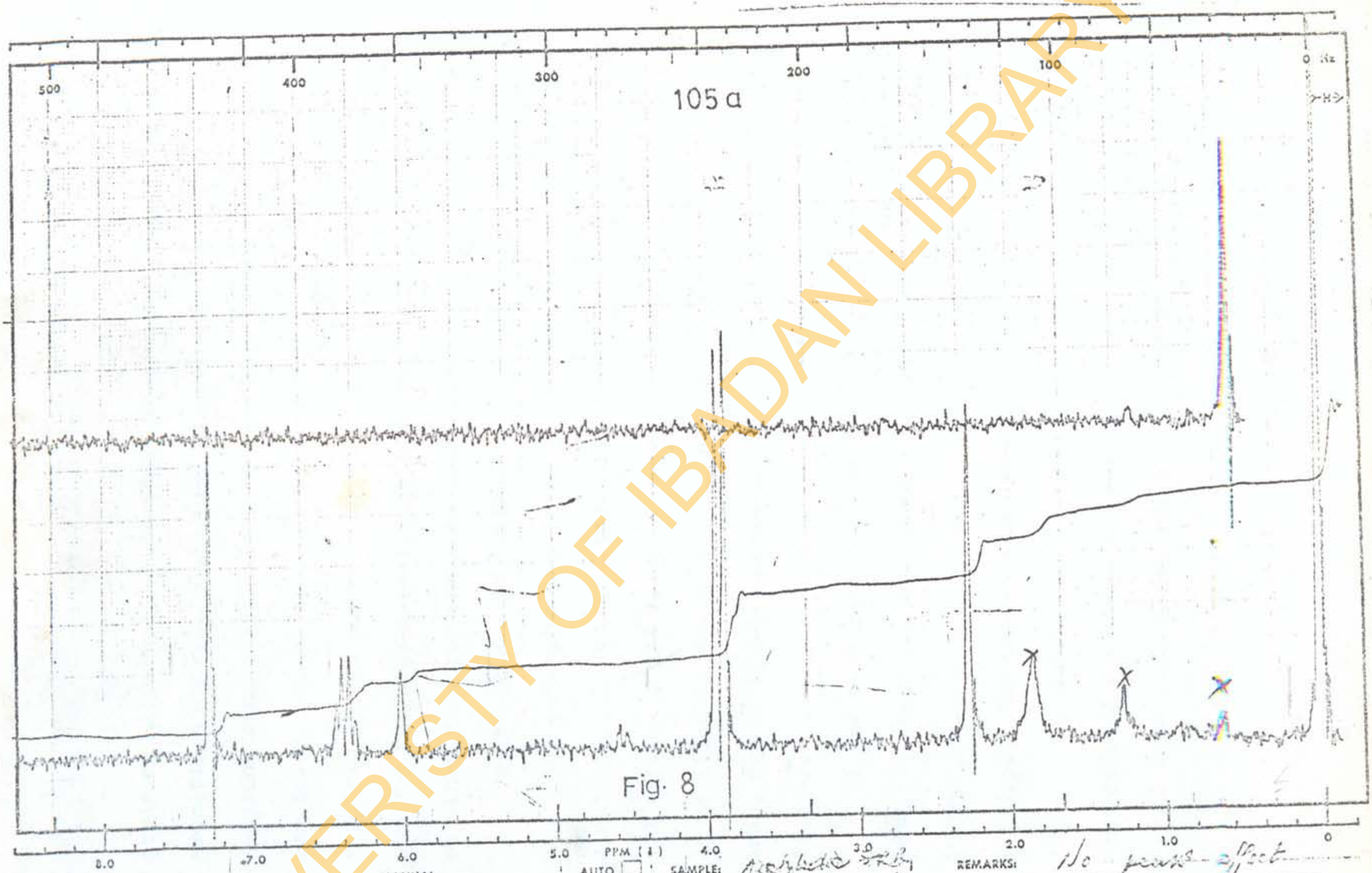


Fig. 8

SWEEP OFFSET (Hz): 450
SPECTRUM AMPLITUDE: 40
INTEGRAL AMPLITUDE: 4.0
SPINNING RATE (RPS): 32

MANUAL
SWEEP TIME (SEC): 50 | 250
SWEEP WIDTH (Hz): 25 | 50 | 100 | 250 | 500
FILTER: 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8
RF POWER LEVEL: 0.05

AUTO
(250)
(500)
(2)
(.05)

SAMPLE: Acetylated [illegible] ([illegible]) ([illegible])
SOLVENT: $CDCl_3$

REMARKS: No [illegible] effect

60 MHz NMR SPECTRUM NO.

DATE: [illegible]

OPERATOR: J.O. Adeboye

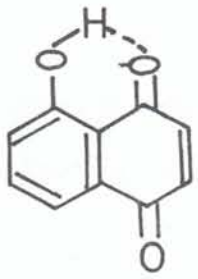
group on γ-pyrone), δ2.40 (3H, s; acetate), δ3.87 (3H, s; aromatic methoxy), δ5.93 (1H, s, proton at 3-position of γ-pyrone ring), δ6.51 (1H, d; aromatic proton, J = 2Hz) and δ6.68 (1H, d; aromatic proton, J = 2Hz). The NMR spectrum of the acetate 125 has not been reported before now and the signal assignments suggested above are based on other reported chromone spectra.

Treatment of SRB_1 with excess dimethylsulphate and anhydrous potassium carbonate in dry acetone afforded the dimethylether of SRB_1. The dimethylether had a m.p. 122-123°;(lit.[82], 124°). The NMR spectrum (FIG.8) showed, in addition to the proton signals of the parent compound, SRB_1, two methoxyl signals at δ3.88 (3H, s) and δ3.93 (3H, s).

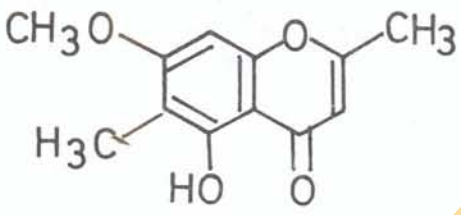
It had been reported[79] that majority of the naturally occuring chromones contained a methyl group at C-2 which, in the NMR spectrum of 2-methylchromone 127 gave a singlet at δ2.36. The location of this signal varied with different compounds but the presence of this substituent was observed to have a marked effect on the shape of the signal from C-3 proton, which was broadened in most spectra

and may even be doubled. This was exactly the observation in the case of SRB_1, the proton signal at δ6.08 (1H, s) was broad, indicating the presence of one methyl group at 2-position. The location of the methyl at 2-position was further supported by the biogenesis of chromones. The melting point of SRB_1 was in agreement with that of 5,7-dihydroxy-2-methylchromone 97. The reported[52] naturally occuring 5,7-dihydroxy-2-methylchromone 97 had a m.p. 268° (decomp.) while as a synthetic product[75,76], it had a m.p. 278-280°. From these deductions, SRB_1 was said to be identical with 5,7-dihydroxy-2-methylchromone 97. This was confirmed by synthesis.

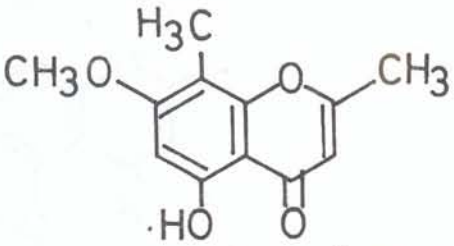
It would be worthwhile to mention in brief what was observed in the first attempt to prepare the dimethylether of SRB_1 by treatment with MeI/Ag_2O in chloroform.[80] This was a method used to methylate the hydrogen-bonded phenolic hydroxyl group of juglone 128. Rather than obtaining the dimethylether of SRB_1, a compound which was identical with eugenitin 129 (m.p. 163°)[78] was obtained.



128



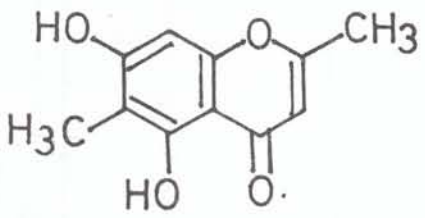
129



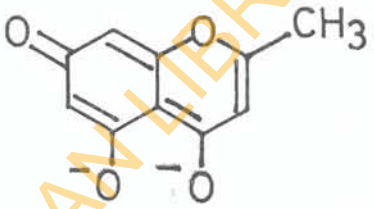
130 Isoeugenitin (m.p. 148°)

It had a m.p. 156-157°, but because of the presence of traces of the 8-methylisomer, that is, isoeugenitin 130 (m.p. 148°)[78], part of the product melted at 143-144°. The presence of the 8-methylisomer was responsible for the lowering of the melting point of eugenitin 129. Eugenitin 129 has been reported[76] to be difficult to synthesize. This is because at one point or another, the acid conditions used in the usual techniques permitted the methyl group to attain the favoured 8-position. It was however obtained by directly heating 5,7-dihydroxy-2-methylchromone 97 with methyl iodide and sodium ethoxide[81]. Since this useful result seemed to conflict with the general tendency of eugenitol 131 to rearrange[78] (at least in acid media) into the 8-methylisomer and that of 7-hydroxy-

coumarins to suffer substitution at the 8-position, the following explanation was suggested.[78]



131



132

The conditions used for C-alkylation at the 6-position were sufficiently basic to ensure ionization of both hydroxyl groups and the ion produced would have structure 132 as a very important cannonical form. In this, the charge at what was the 5-hydroxyl group would presumably be much less diffuse than that originating in the 7-hydroxyl group because it was less easily shared by the carbonyl group and so it would be exploited to form new bond with incoming methyl group which could attach itself at the 6-position rather than 8-position. Because of the comparative basic conditions leading to the C-alkylation

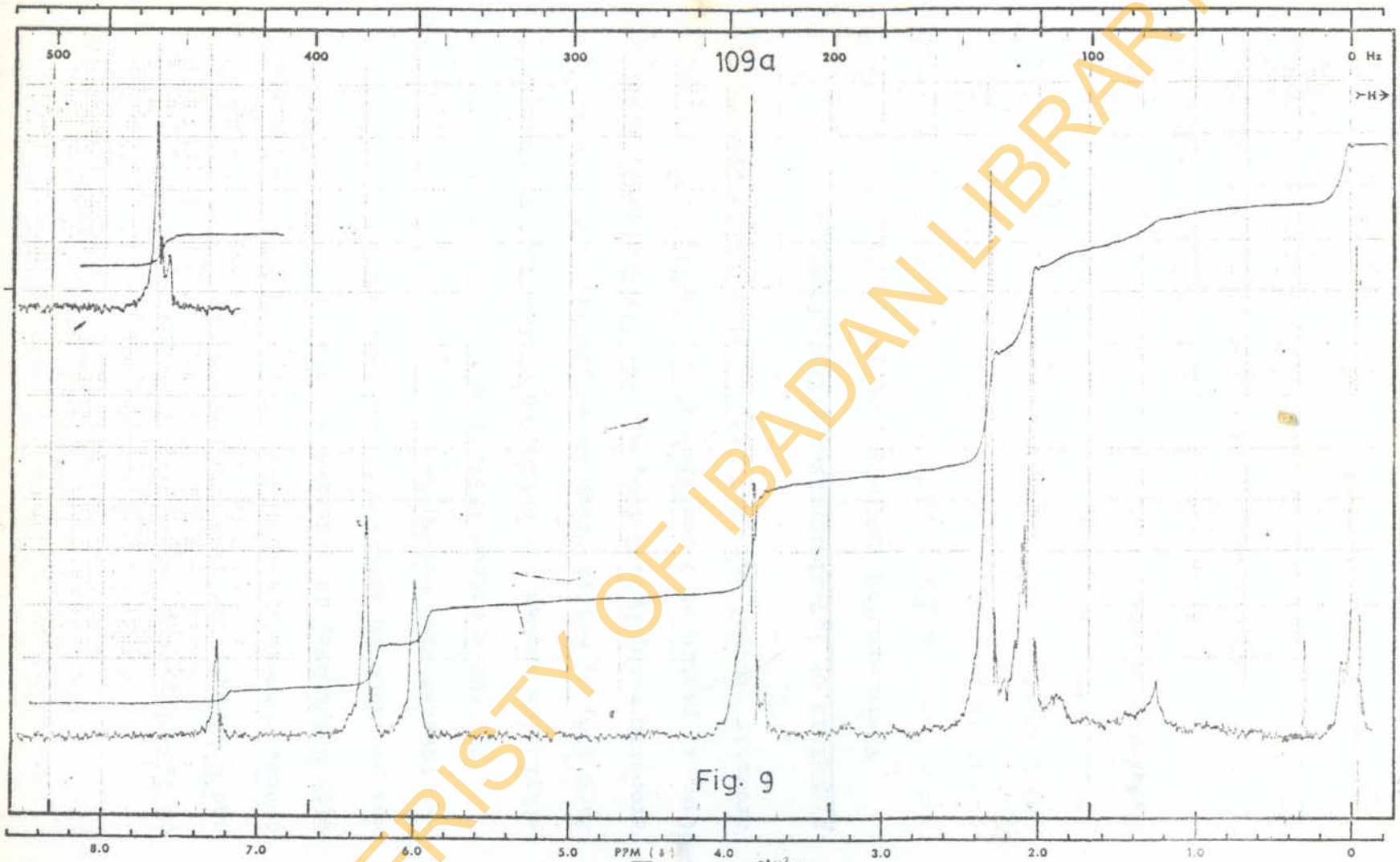


Fig. 9

SWEEP OFFSET (Hz): 200
SPECTRUM AMPLITUDE: 35
INTEGRAL AMPLITUDE: 4·0
SPINNING RATE (RPS): 32

MANUAL
SWEEP TIME (SEC): 50 250
SWEEP WIDTH (Hz): 25 50 100 250 500
FILTER: 1 2 3 4 5 6 7 8
RF POWER LEVEL: 0·05

AUTO ☐
(250)
(500)
(2)
(.05)

SAMPLE: MBN Alkylated MB1 (tuguenitin)

REMARKS:

SOLVENT: CDCl3

DATE: 5/5/78

OPERATOR: J. O. Adeboye

60 MHz NMR SPECTRUM NO.

of 5,7-dihydroxy-2-methylchromone 97, using MeI/EtONa and MeI/Ag_2O, the same explanation might hold in both cases. However, while only the 6-position was alkylated with MeI/EtONa, it appeared a little of the 8-methylisomer was produced in addition to the 6-methylisomer when 97 was treated with MeI/Ag_2O.

The NMR spectrum (FIG.9) of the product of methylation/alkylation showed the following proton signals (δppm): 2.08 (3H, s, methyl group on γ-pyrone), 2.35 (3H, s, aromatic methyl), 3.73 (3H, s, aromatic methoxy), 6.03 (1H, s, proton at 3-position of γ-pyrone), 6.35 (2H, s, aromatic proton) and 12.7 (1H, s, disappeared with D_2O).

SYNTHESIS OF 5,7-DIHYDROXY-2-METHYLCHROMONE.

There are two synthetic approaches to 5,7-dihydroxy-2-methylchromone 97. The first goes through 5,7-dimethoxy-2-methylchromone 126, which affords 5,7-dihydroxy-2-methylchromone on demethylation.[82] The second method involves the formation of γ-pyrone ring by treating 2,4,6-trihydroxyacetophenone with acetic anhydride and sodium acetate.

The latter method[83] was employed in the synthesis of 97 because of the easily accessible 2,4,6-trihydroxyaceto-

110 a

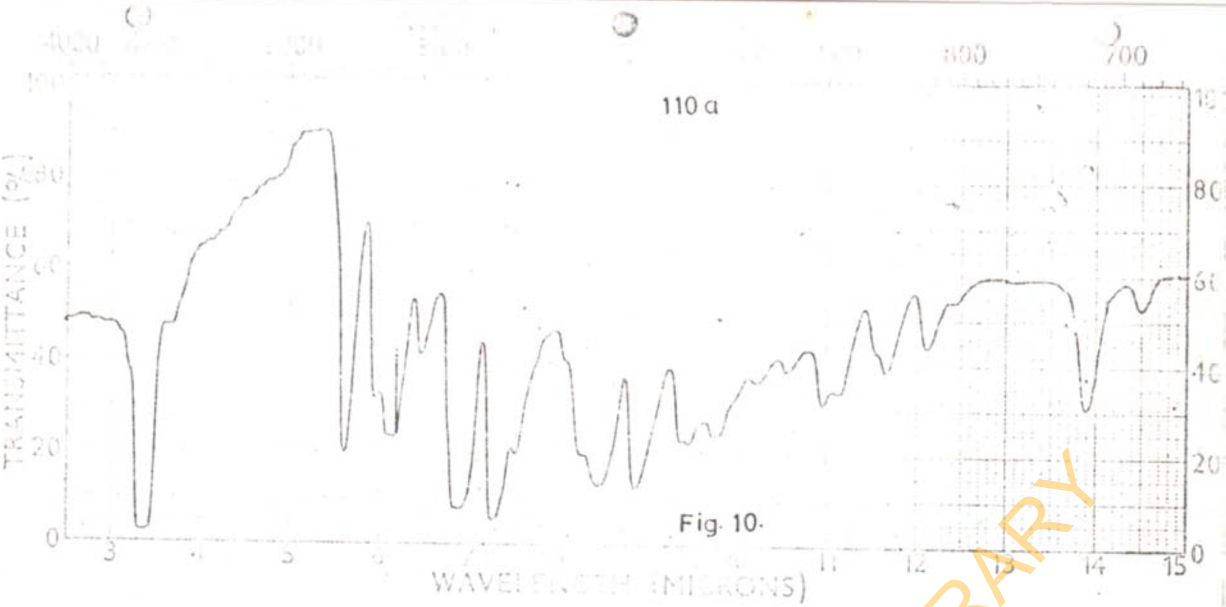
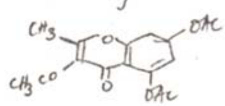


Fig. 10.

SAMPLE Synthesized Acetate Ketone of Nor-eugenin



ORIGIN

PHASE Nujol

SOLVENT

CONC.

CELL PATH

REFERENCE

SCAN SPEED fast SLIT N

OPERATOR ADEBOYE DATE 13-8-79

REMARKS

PART NO. 1375-1.61

PERKIN-ELMER LIMITED, BEACONSFIELD, BUCKS

110 a'

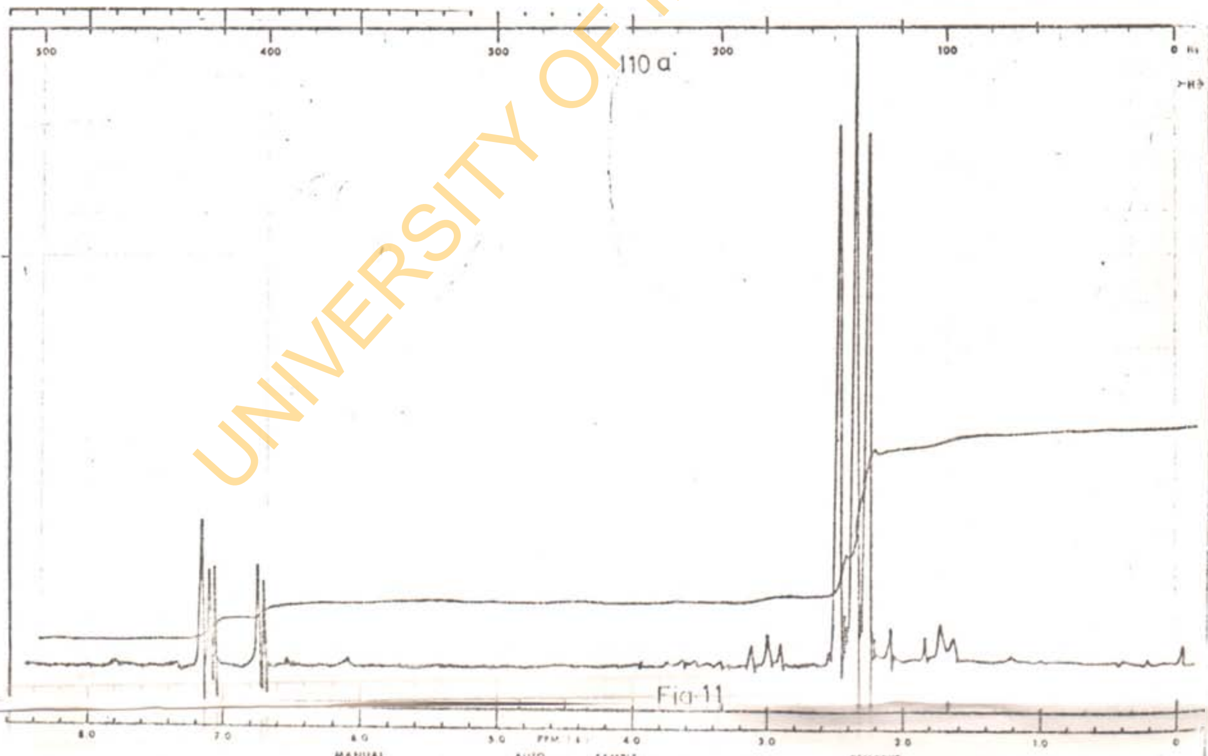


Fig 11

SWEEP OFFSET (Hz)
SPECTRUM AMPLITUDE
INTEGRAL AMPLITUDE
SPINNING RATE (RPS)

MANUAL
SWEEP TIME (SEC)
SWEEP WIDTH (Hz)
FILTER
RF POWER LEVEL

AUTO
(250)
(500)
(.2)
(.05)

SAMPLE

SOLVENT $CDCl_3$

REMARKS

DATE

OPERATOR J. O. ADEBOYE

60 MHz NMR SPECTRUM NO.

110b

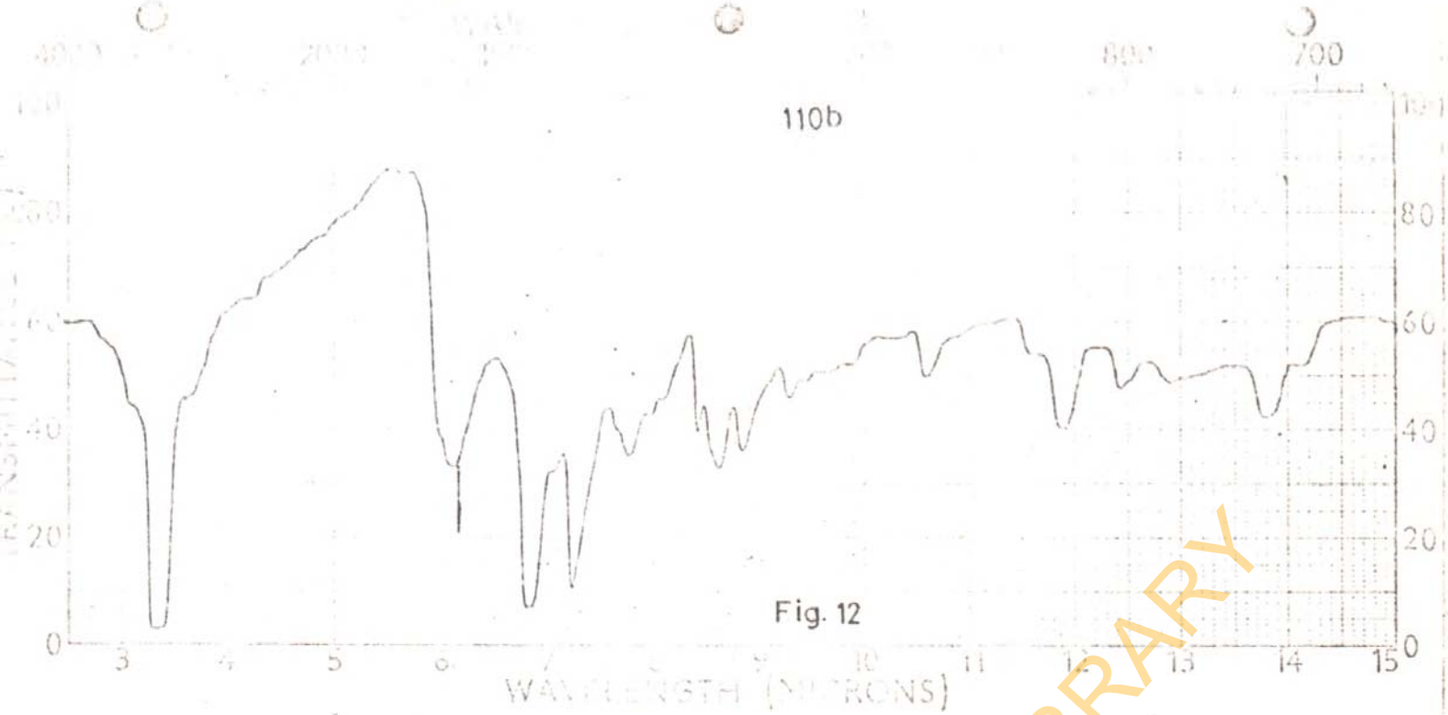
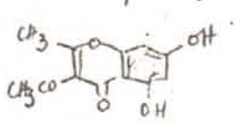


Fig. 12

SAMPLE Synthesized Ketone



Nujol

SCAN SPEED FAST SLIT N.

OPERATOR ADEBOYE DATE 13-8-77

REMARKS

ORIGIN

110b

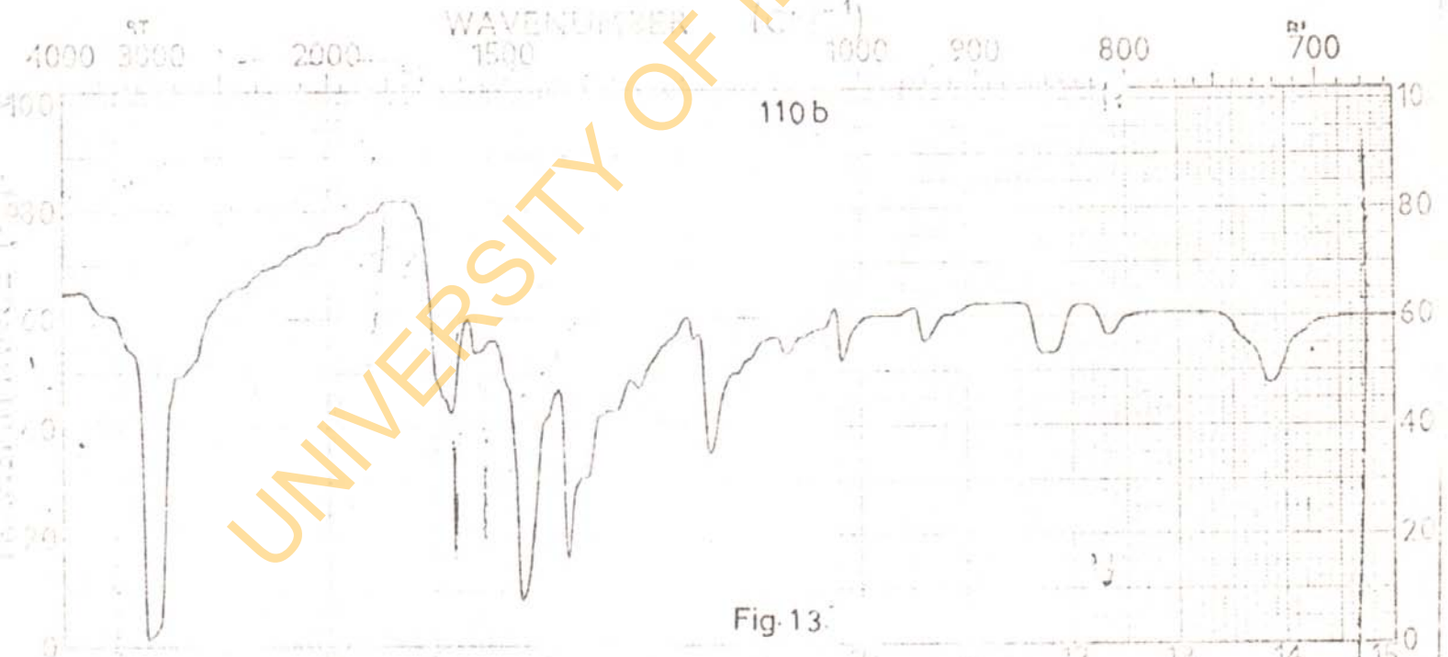
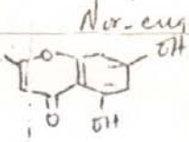


Fig. 13

Synthesized Nor-eugenin



Nujol

SCAN SPEED FAST SLIT N.

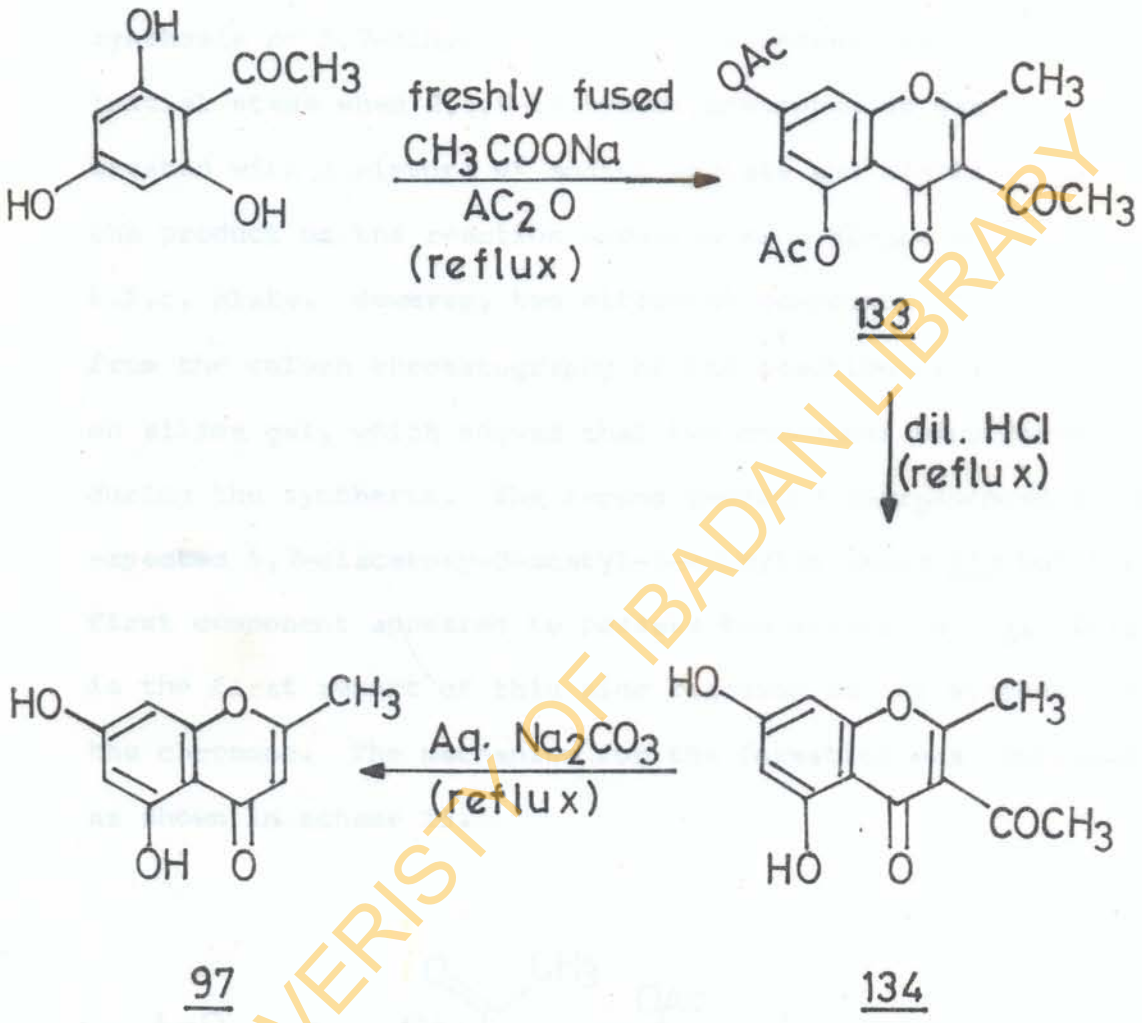
OPERATOR ADEBOYE DATE 13-8-77

phenone either commercially or from Hoesch reaction.[96]

Refluxing a mixture of 2,4,6-trihydroxyacetophenone, acetic anhydride and freshly fused sodium acetate gave 5,7-diacetoxy-3-acetyl-2-methylchromone 133, m.p. 129-130°, (lit[83], 129-131°). IR (FIG. 10) and NMR (FIG.11).

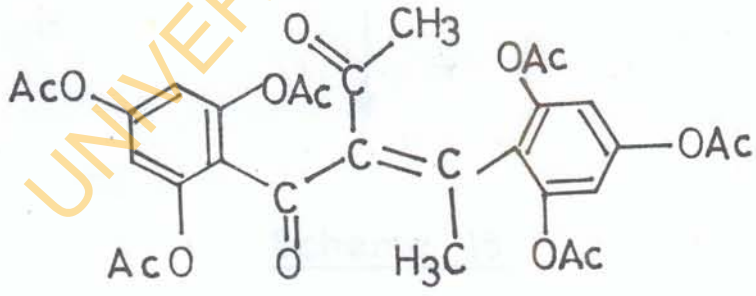
Treatment of the diacetate 133 with dilute hydrochloric acid afforded 3-acetyl-5,7-dihydroxy-2-methylchromone 134, m.p. 250-251°, (lit.[83], 250-251°). The infra-red spectrum (FIG. 12) showed the characteristic carbonyl absorption at 1660cm^{-1}.

3-Acetyl-5,7-dihydroxy-2-methylchromone 134 was digested in aqueous sodium carbonate solution by boiling under reflux, followed by acidification to give 5,7-dihydroxy-2-methylchromone (noreugenin) 97, m.p. 280-282°; (lit.[83], 281-282°). The IR spectrum (FIG. 13) was identical with the IR spectrum of the naturally occurring noreugenin. The mass spectrum gave the molecular ion as 192 which agreed with the expected value. The synthetic procedure is represented in scheme 14.

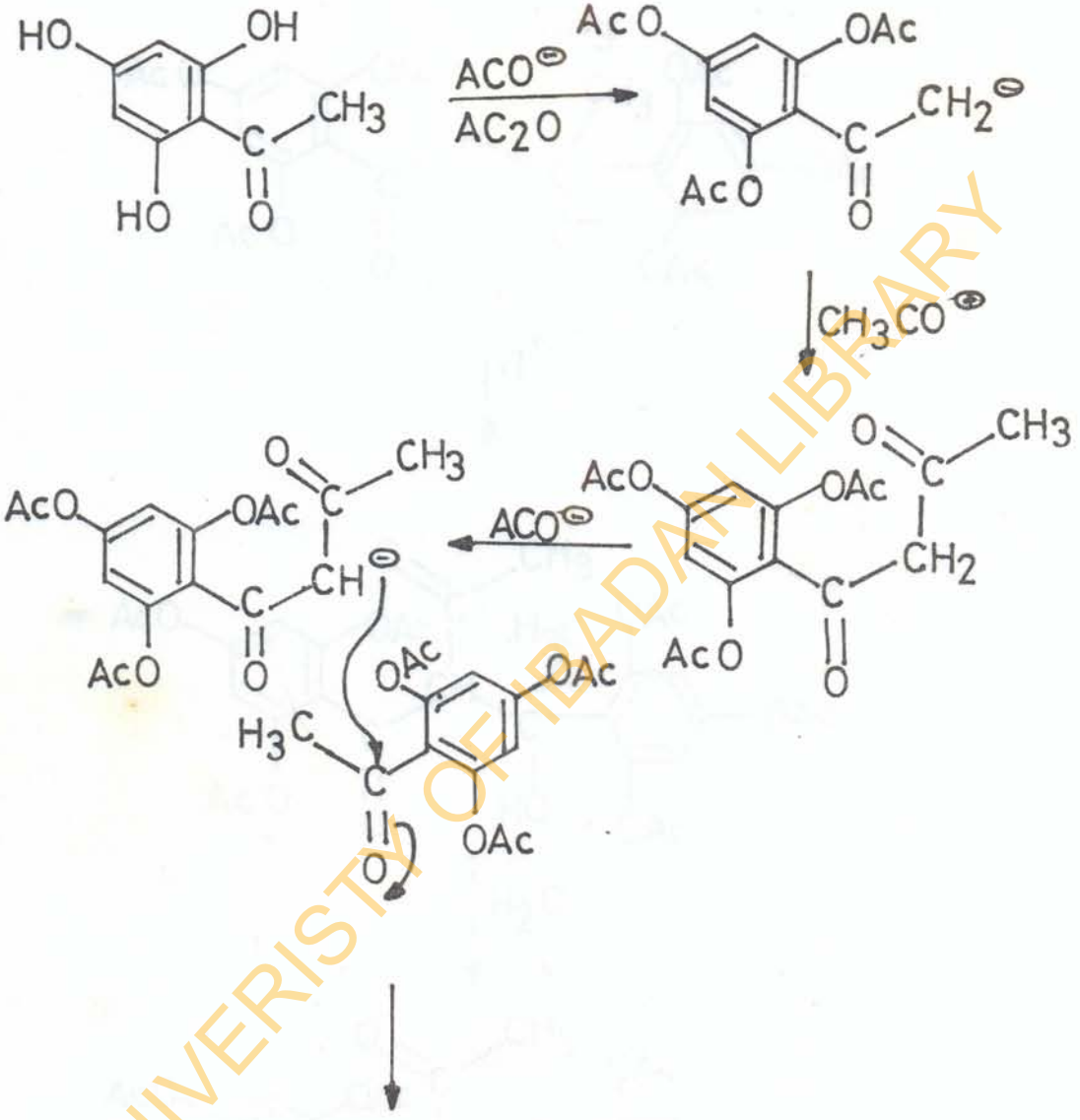


Scheme 14

There was an interesting observation during the synthesis of 5,7-dihydroxy-2-methylchromone. At the initial stage when 2,4,6-trihydroxyacetophenone was treated with a mixture of sodium acetate and acetic anhydride, the product of the reaction appeared as a single spot on the t.l.c. plate. However, two different compounds were recovered from the column chromatography of the reaction product on silica gel, which showed that two compounds were formed during the synthesis. The second compound corresponded to the expected 5,7-diacetoxy-3-acetyl-2-methylchromone 133 but the first component appeared to possess the structure 135. This is the first report of this side reaction in the synthesis of the chromone. The mechanism for the formation was explained as shown in scheme 15.

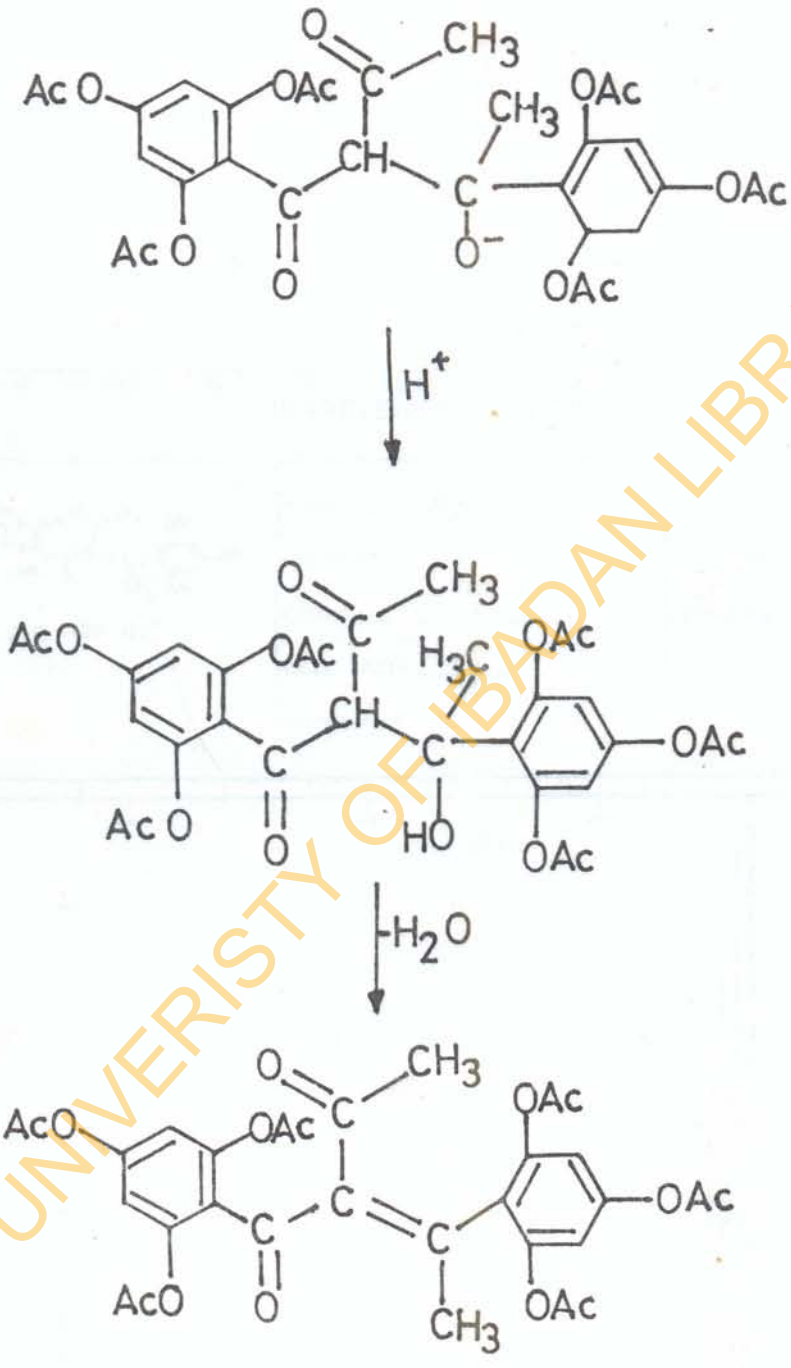


135



Scheme 15

Scheme 15 contd.



135

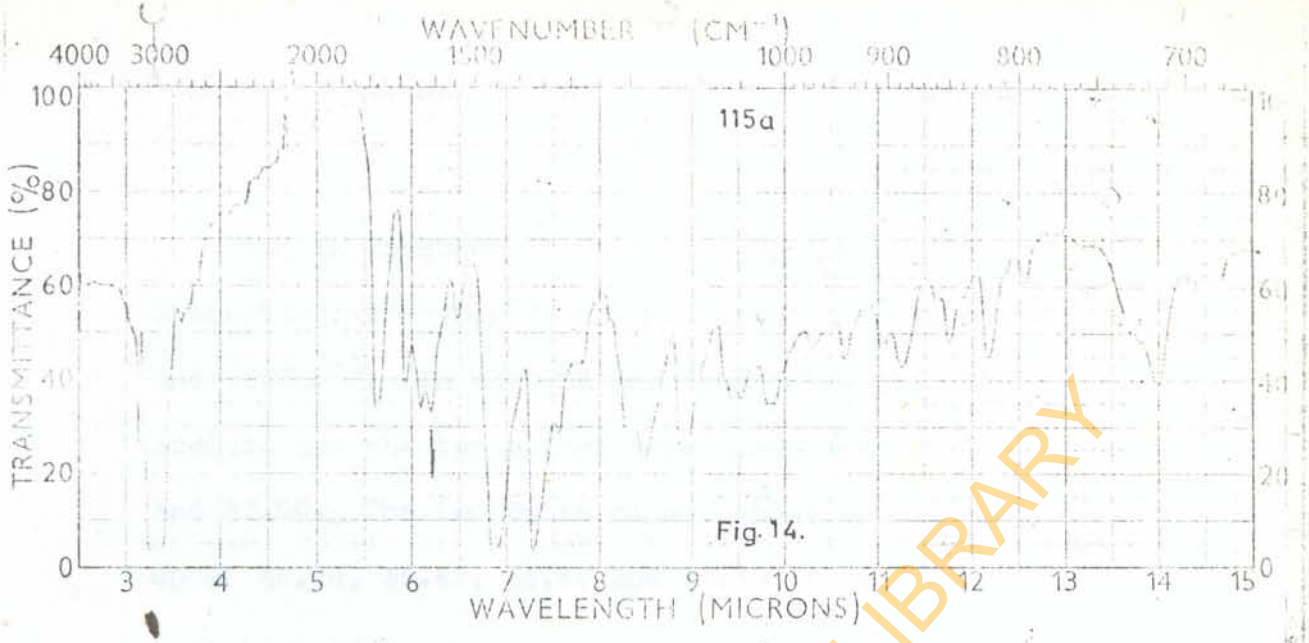
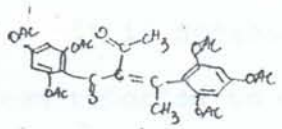


Fig. 14.

SAMPLE 

m.p. 120-122°

135

ORIGIN

PHASE Nujol

SOLVENT

CONC.

CELL PATH

REFERENCE

SCAN SPEED FAST SLIT NORMAL

OPERATOR ADEBOYE DATE 7/5/81

REMARKS

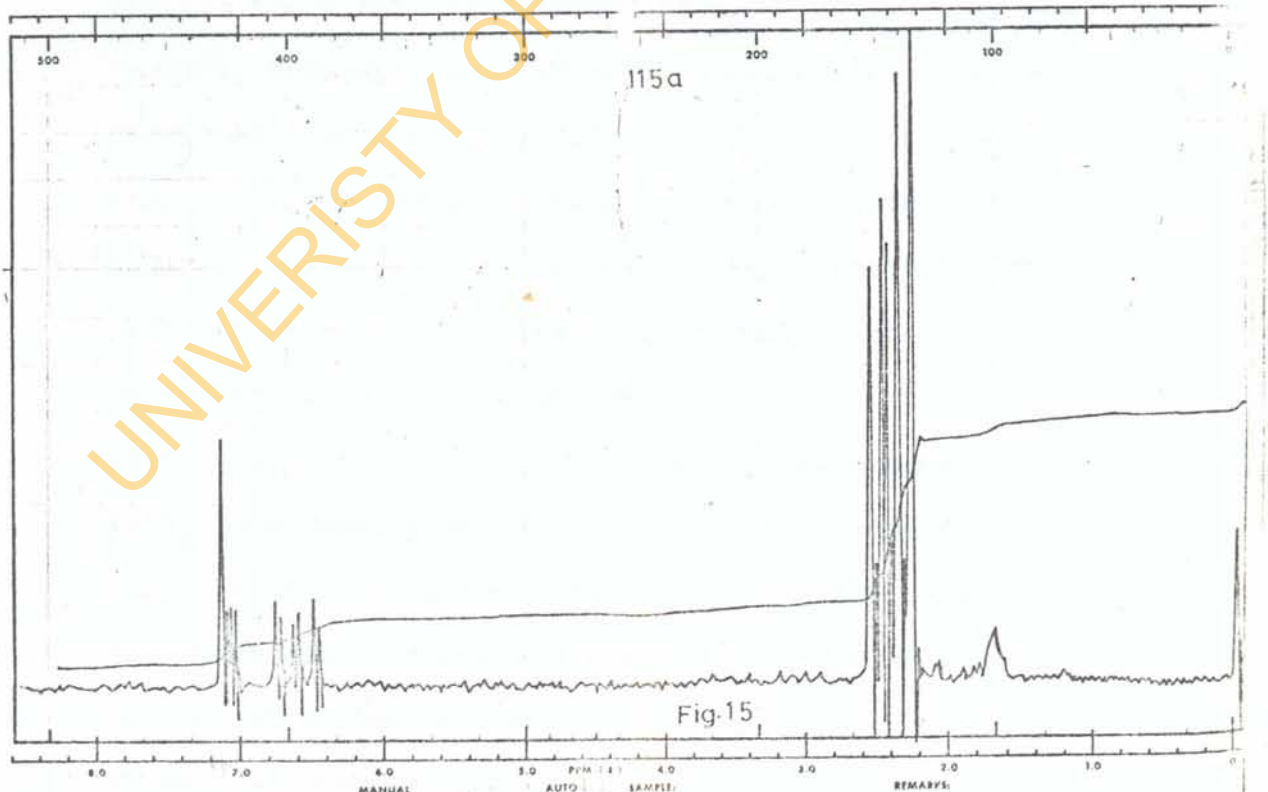


Fig. 15

The IR spectrum (FIG.14) showed the acetate absorption at 1770cm^{-1} and the carbonyl bands at 1680cm^{-1} and 1640cm^{-1}. In the NMR spectrum (FIG. 15), the six acetate and the two methyl groups appeared between δ2.24 and δ2.50. The two pairs of meta-coupled protons showed up at δ6.46, δ6.62, δ6.80 and δ7.10 as one proton doublets each (J = 2Hz).

It is necessary to mention that, although SRB_1 has been reported to occur as a natural product[52] and was well known as synthetic product[75,76], much work was done on it for two important reasons. Since in the NMR spectra taken both in d_6-DMSO and d_6-CH_3COCH_3, only one phenolic hydroxyl group absorption was observed in each case, which occured at δ12.7 (1H, s, disappeared with D_2O), there was doubt as to whether SRB_1 was identical with 5,7-dihydroxy-2-methylchromone. This necessitated the preparation of many of its derivatives. Secondly, SRB_1 was synthesized because it was discovered in the course of this work that two chromone alkaloids, schumannificine (SRB_4) and N-methylschumannificine (SRB_3) were related to SRB_1 and the latter could be a good starting point in the synthesis of these alkaloids. The relationship between the two alkaloids and SRB_1 is discussed in detail under "Chromone Alkaloids".

CHROMONE ALKALOIDS.

(I) SCHUMANNIFICINE (SRB_4)

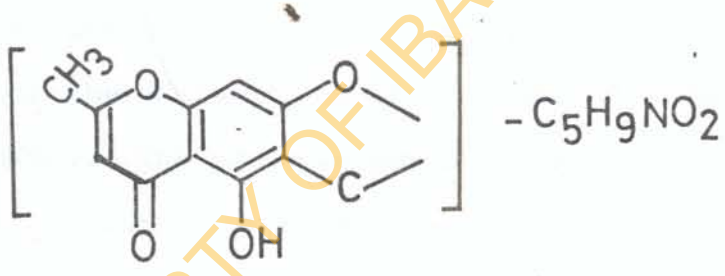
The compound, SRB_4, hereby named schumannificine, which had a m.p. 234° (recovered from MeOH) was shown to contain nitrogen (microanalysis) with molecular formula $C_{16}H_{15}NO_6$. It gave a positive alkaloid test with the Dragendorff's reagent. It also gave a positive ferric chloride test so it was phenolic.

Much of the information on the structure of schumannificine (SRB_4) came from the alcoholic potassium hydroxide hydrolysis of the alkaloid and the comparison of its mass, ultra-violet, and infrared spectra with those of 5,7-dihydroxy-2-methylchromone 97.

Alcoholic potassium hydroxide hydrolysis of schumannificine gave a compound which was identical (m.p., mixed m.p. and spectra) with 5,7-dihydroxy-2-methylchromone 97. This established the chromone portion of the constitutional formula of schumannificine. The nitrogen-containing portion of the alkaloid could not be isolated from the reaction mixture.

In the NMR spectrum of the hydrolysis product, the two meta-coupled protons appeared at δ6.18 and δ6.28 as a pair of doublets (J=2Hz) as expected, whereas there was no meta coupling in the starting material, that is, SRB_4, but only one aromatic proton was observed at δ6.66. This then suggested that either the 6- or the 8-position of 5,7-dihydroxy-2-methylchromone was involved in a bond with carbon. However, it is a well established fact that when phloroglucinol derivatives, and chromones are refluxed with methyl iodide under basic conditions, C-alkylation usually takes place[81,84,85]. In the case of 5,7-dihydroxy-2-methylchromone, the 6-position is more susceptible to C-alkylation than the 8-position.[78,81] Since on refluxing SRB_4 and 5,7-dihydroxy-2-methylchromone separately with MeI/Ag_2O, no C-alkylation was observed in the former while alkylation at C-6 took place in the latter, it was reasonable to make the deduction that the 6-position in SRB_4 was bonded to a carbon atom. In the NMR spectra of both natural and synthetic chromones reported by Badawi and Fayez[79], that of 5,7-dihydroxy-2-methylchromone was run in deuteropyridine. The chemical shift value for the hydrogen at the 6-position was δ6.58 while that at the

8-position was δ6.67. In the NMR spectra of both SRB_4 and SRB_3 taken in deuteropyridine, the aromatic proton appeared at δ6.66 which was very close to that of the hydrogen at the 8-position of the chromone. This further strengthened the arguement that the 8-position was bonded to hydrogen while the 6-position was bonded to a carbon atom. On the basis of the hydrolysis and the above deductions, the partial structure 136 was assigned for the compound.

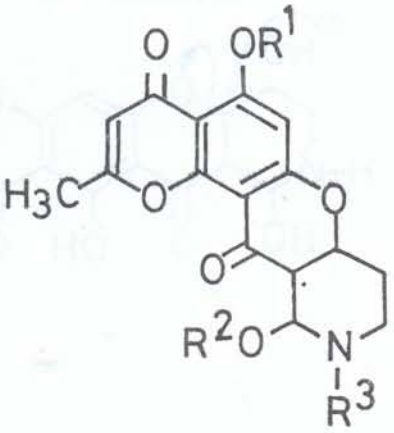


136

Comparison of the NMR spectra of SRB_4 and 5,7-dihydroxy-2-methylchromone 97 revealed that both the methyl group and the one proton at 2- and 3- positions respectively of γ-pyrone ring were still present in SRB_4. The UV and IR spectra of schumannificine (SRB_4) gave characteristic chromone absorptions.

It was observed that the diacetate and the dimethylether derivatives of SRB_4 were formed when SRB_4 was treated with a mixture of pyridine/Ac_2O and MeI/Ag_2O which suggested that there were two hydroxyl groups in schumannificine (SRB_4). Since samples sent for mass spectra and microanalysis were lost in transit, no report of mass spectra and elemental analysis could be given here. The two acetate proton signals had different chemical shift values; δ2.05 (3H, s, $OCOCH_3$) and δ2.36 (3H, s, $-OCOCH_3$). The three proton singlet at δ2.36 was assigned to the acetate attached to an aromatic ring while the other was for the acetate attached to a non-aromatic ring. Similar thing was observed in the NMR spectrum of the dimethylether of schumannificine (SRB_4). The aromatic and the non-aromatic methoxy protons gave proton absorptions at δ3.90 (3H, s) and δ3.40 (3H, s) respectively. One proton signal (1H,d) which appeared at δ5.7 in the dimethylether derivatives shifted downfield to δ6.93 in the diacetate and monoacetate derivatives of SRB_4. This proton was suggested to be at the base of one of the hydroxyl groups in the starting material, that is, SRB_4. The doublet nature of the proton showed that there could only be one proton on the carbon adjacent to the proton. The proton was then located on a carbon lying between tertiary carbon and the nitrogen atom. Though the isomeric structure 137 could not be completely ruled out, the linear structure 138 was proposed for

schumannificine (SRB_4) by considering the above reasons discussed in connection with the chemical shift of the only one aromatic proton observed.

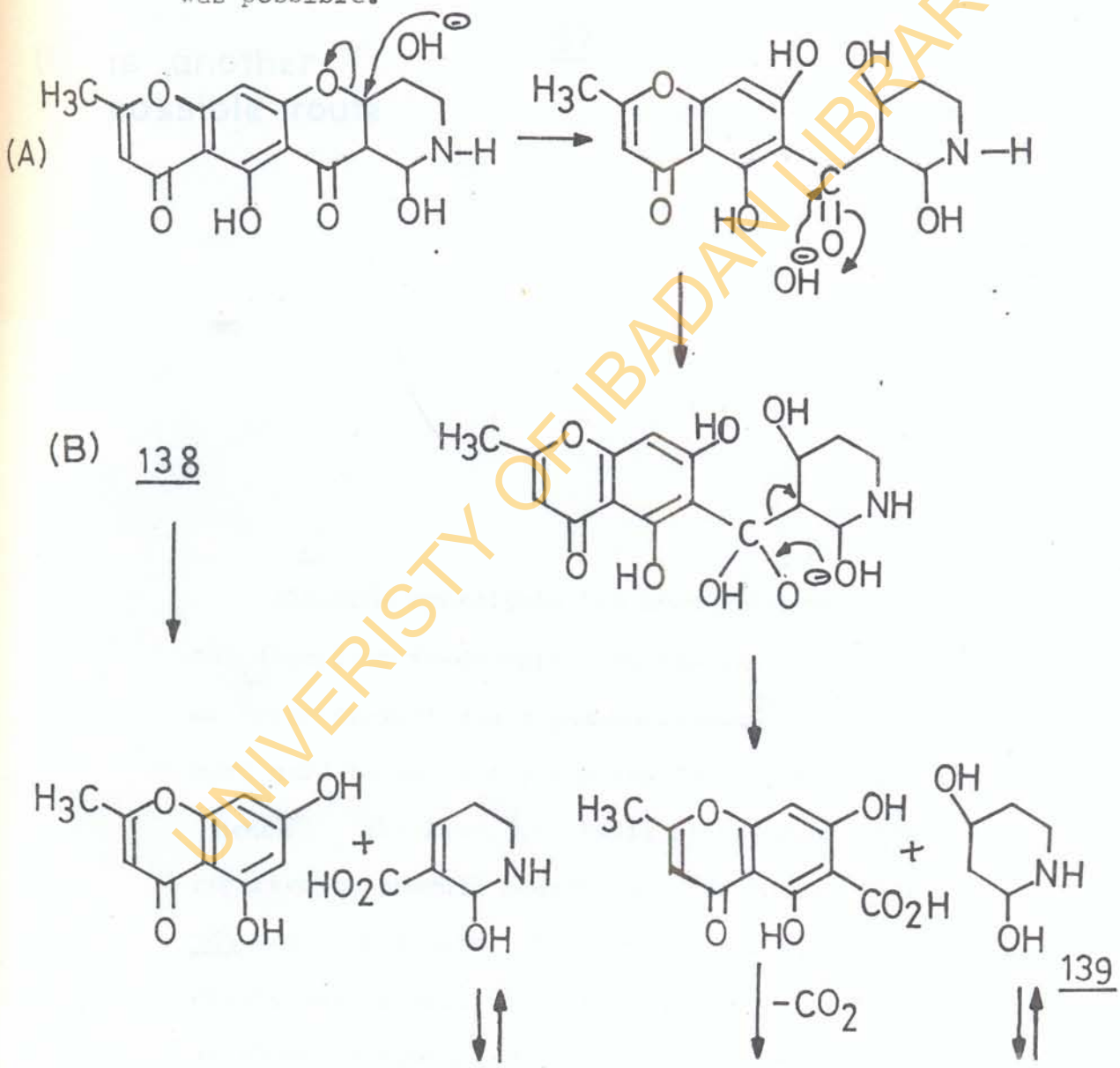


137 ; $R^1 = R^2 = R^3 = H$

138 ; $R^1 = R^2 = R^3 = H$

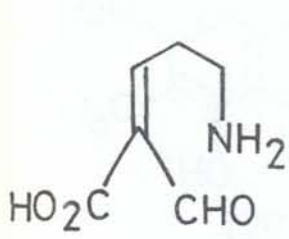
On the basis of the above working structure, the probable mechanism for the alkaline hydrolysis of SRB_4 was proposed in scheme 16. The mechanism explains the formation of 5,7-dihydroxy-2-methylchromone. The nitrogen-containing portion 139 of the hydrolysis reaction is a secondary amine, so it is not surprising that it could not

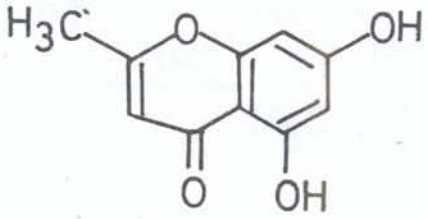
be isolated from the aqueous acidic medium and even after acidification because of the formation of secondary ammonium salt. Its further decomposition as shown in the scheme was possible.

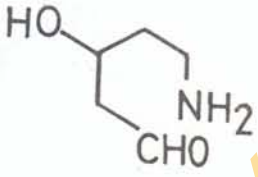


Scheme 16

Scheme 16 contd.

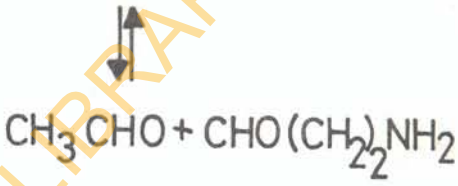




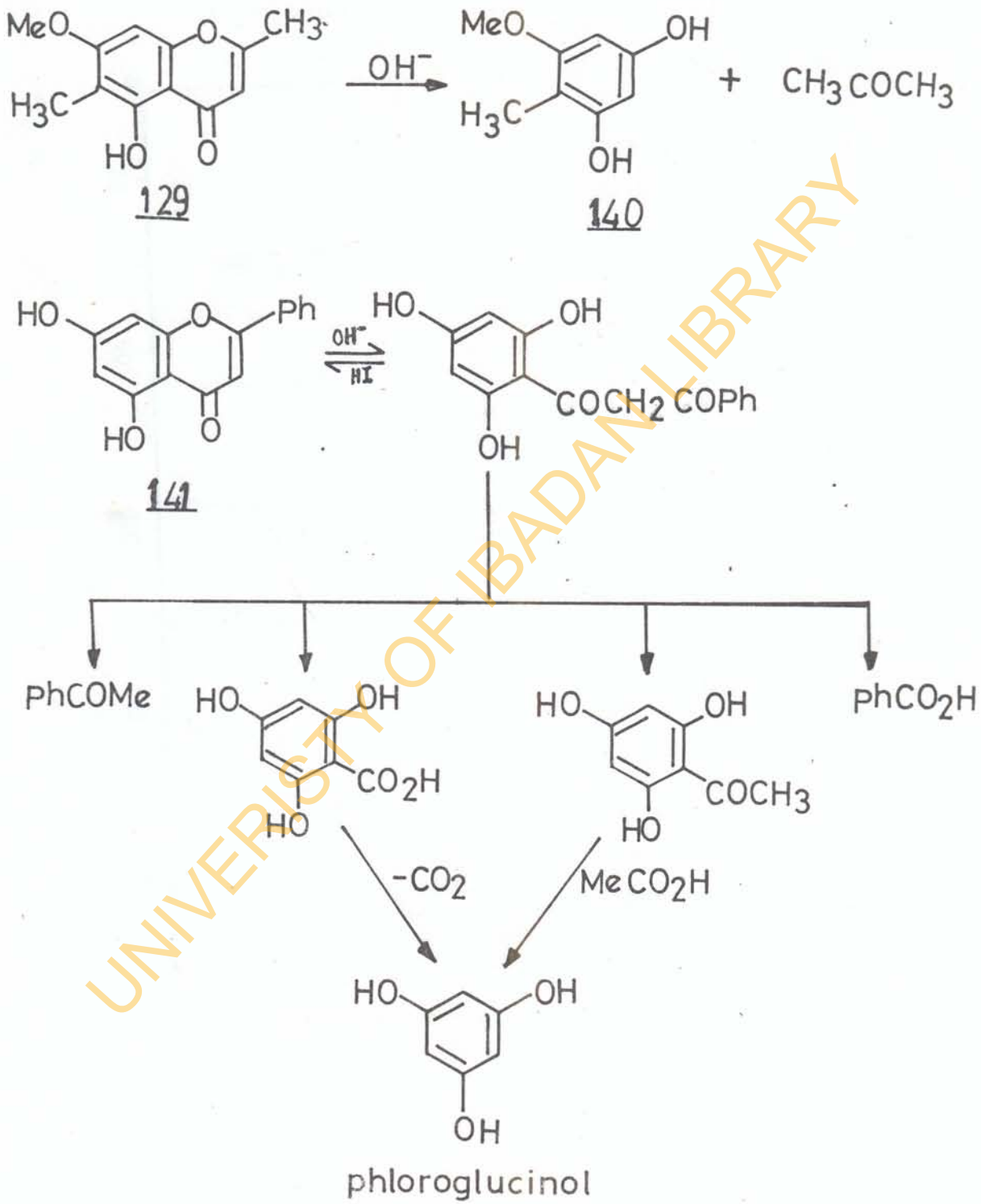


97

(B) is another possible route



Alkaline hydrolysis has been regarded as one of the important fundamental methods in structural studies of polyhydroxyflavones and chromones.[78] Acyl substituents were said to be easily removed from a phloroglucinol residue, hence alkaline hydrolysis of eugenitin 129 was reported to produce acetone and the phenol 140. When chrysin 141 was heated with potassium hydroxide, it gave phloroglucinol, acetic acid, benzoic acid and a little acetophenone as shown in scheme 17. All these reactions mentioned above supported the proposed mechanism in scheme 16.



Scheme 17

124a

SCHUMANNIFICINE

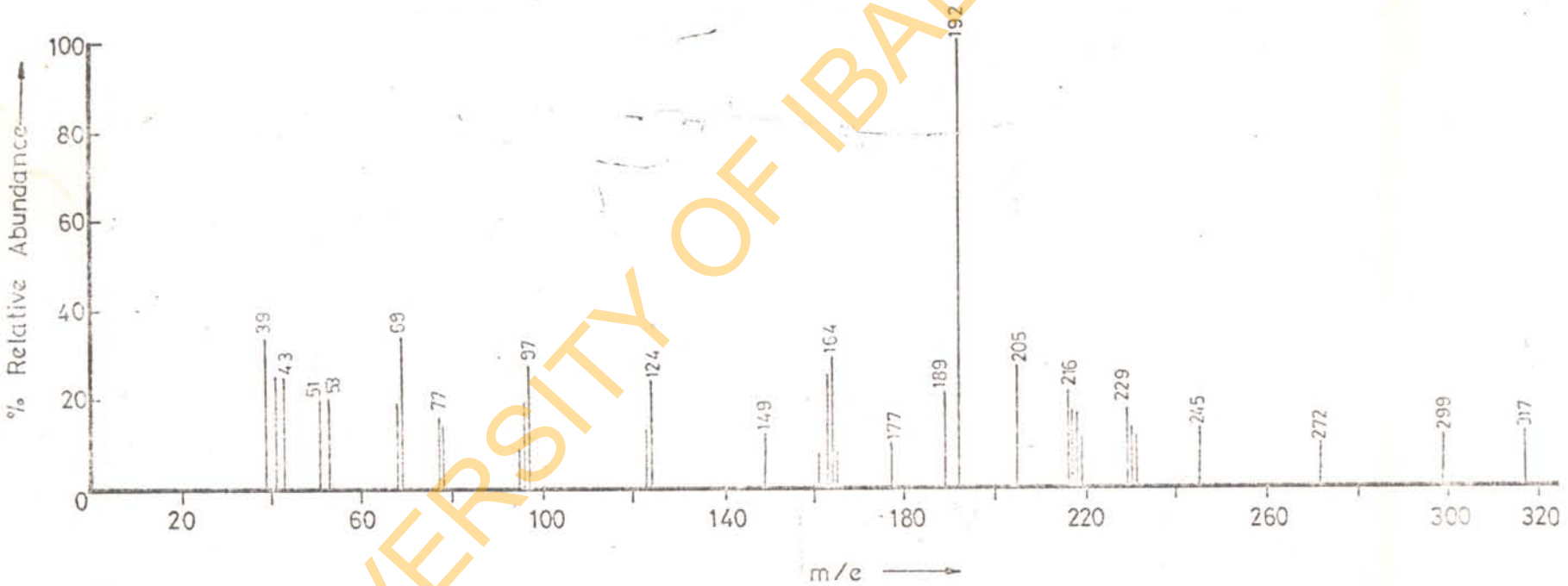


Fig. 15

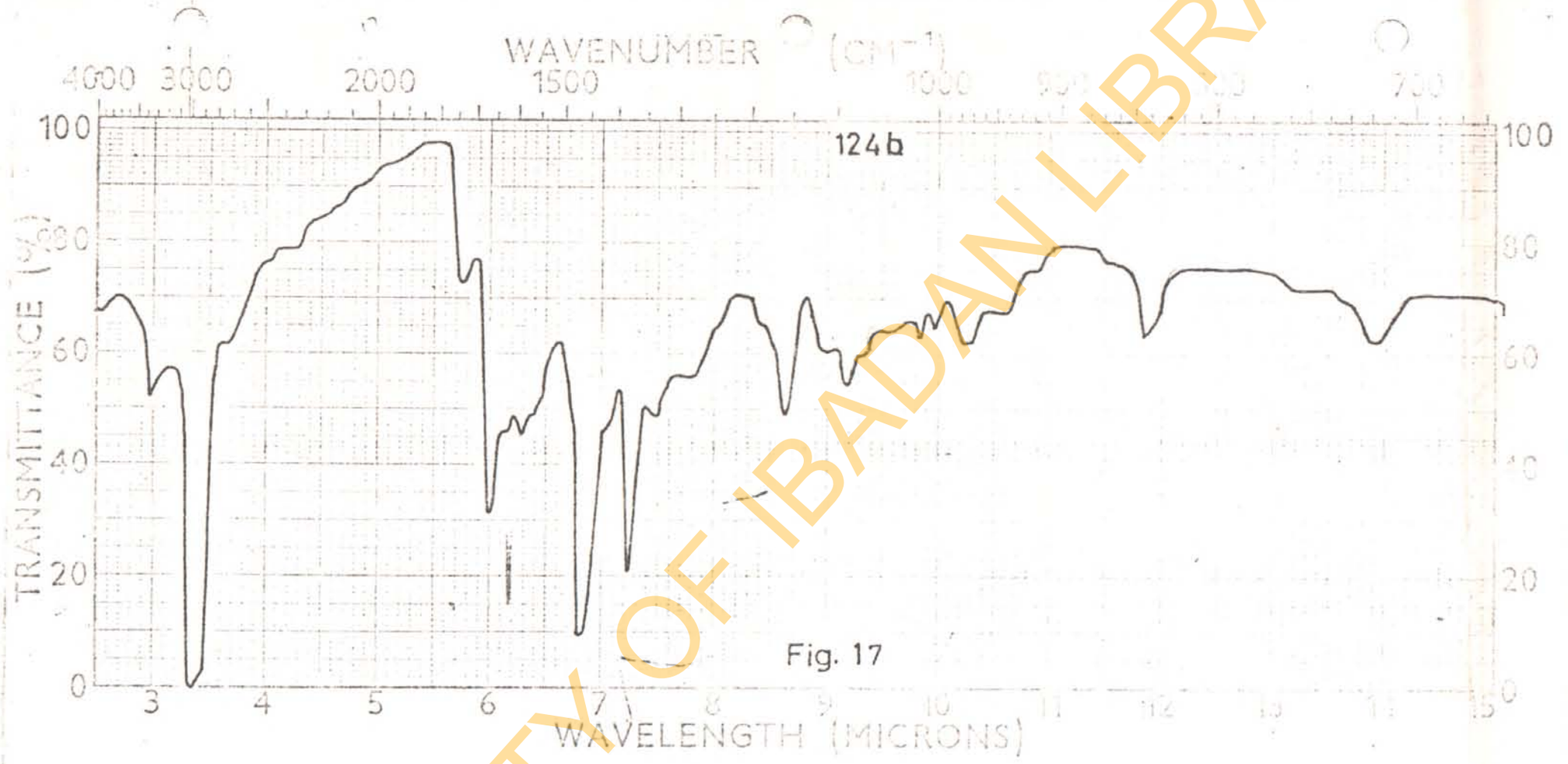


Fig. 17

SAMPLE SRB4

PHASE Nujol

SOLVENT

CONC.

CELL PATH

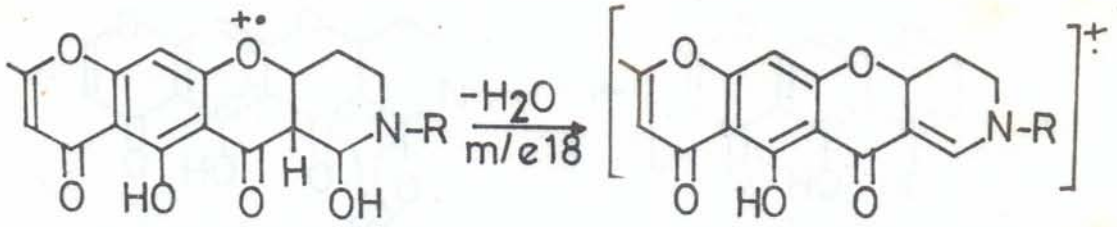
REFERENCE

Fast Normal

J.O. Adetoye 25/10/77

The mass spectrum (FIG. 16) of schumannificine (SRB_4) indicated a parent peak at M^+ 317 (12.1%) which agreed with the molecular formula. The following prominent peaks were observed with the percentage relative abundances shown in brackets: 299 (11.3), 271 (9.7), 245 (12.9), 231 (11.3), 219 (11.3), 218 (16.1), 217 (17), 205 (27.4), 192 (100-base peak), 189 (21), 177 (9.7), 164 (29), 163 (25.8), 149 (12.1), 124 (24.2), 97(26.6), 96 (18.6) and 69 (33.9). Comparison of the mass spectra of 5,7-dihydroxy-2-methylchromone 97 and those of the alkaloids, schumannificine (SRB_4) and N-methylschumannificine (SRB_3) led to the following fragmentation patterns being proposed for the two alkaloids. These are shown in schemes 18a, 18b, and 18c. The order of arrangement of the fragment ions shown in the schemes was dictated by the various observed masses of the fragments.

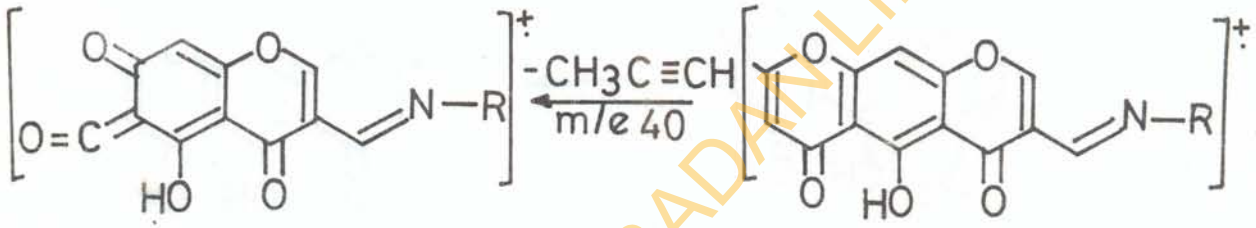
The infrared spectrum of SRB_4 (FIG.17) showed bands at 3150cm^{-1} which was assigned to >N-H. The formation of an amide, >N-$COCH_3$ when SRB_4 was treated with a mixture of acetic acid and acetic anhydride in the presence of catalytic amount of p-toluenesulphonic acid, further provided evidence for the existence of >N-H. The band was



Molecular ion ; R = CH_3; m/e 331
R = H ; m/e 317

R = CH_3 ; m/e 313
R = H ; m/e 299

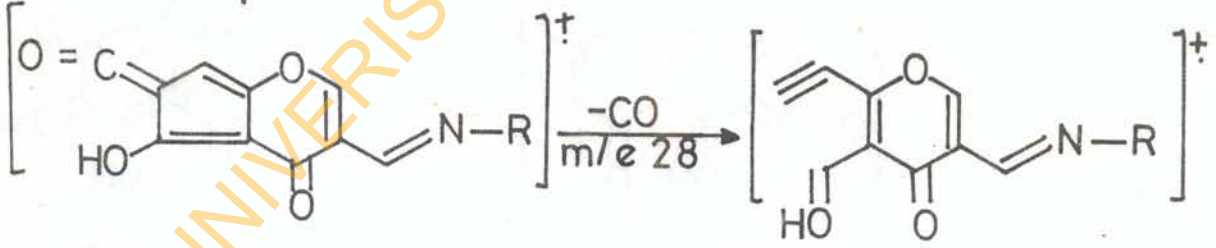
(retro Diels-Alder)
-CH_2=CH_2 m/e 28



R = CH_3 ; m/e 245
R = H ; m/e 231

R = CH_3 ; m/e 285
R = H ; m/e 271

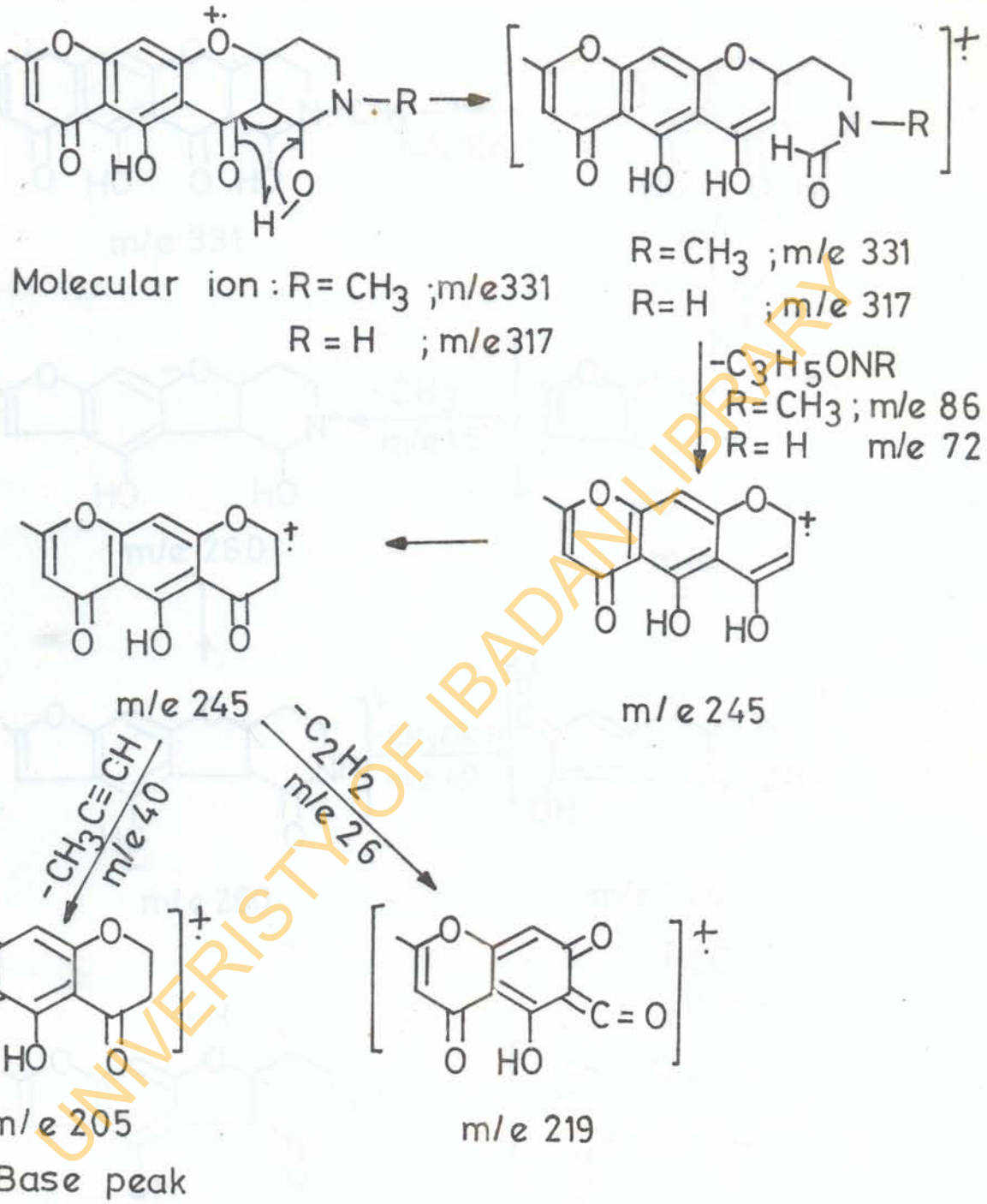
-CO
m/e 28



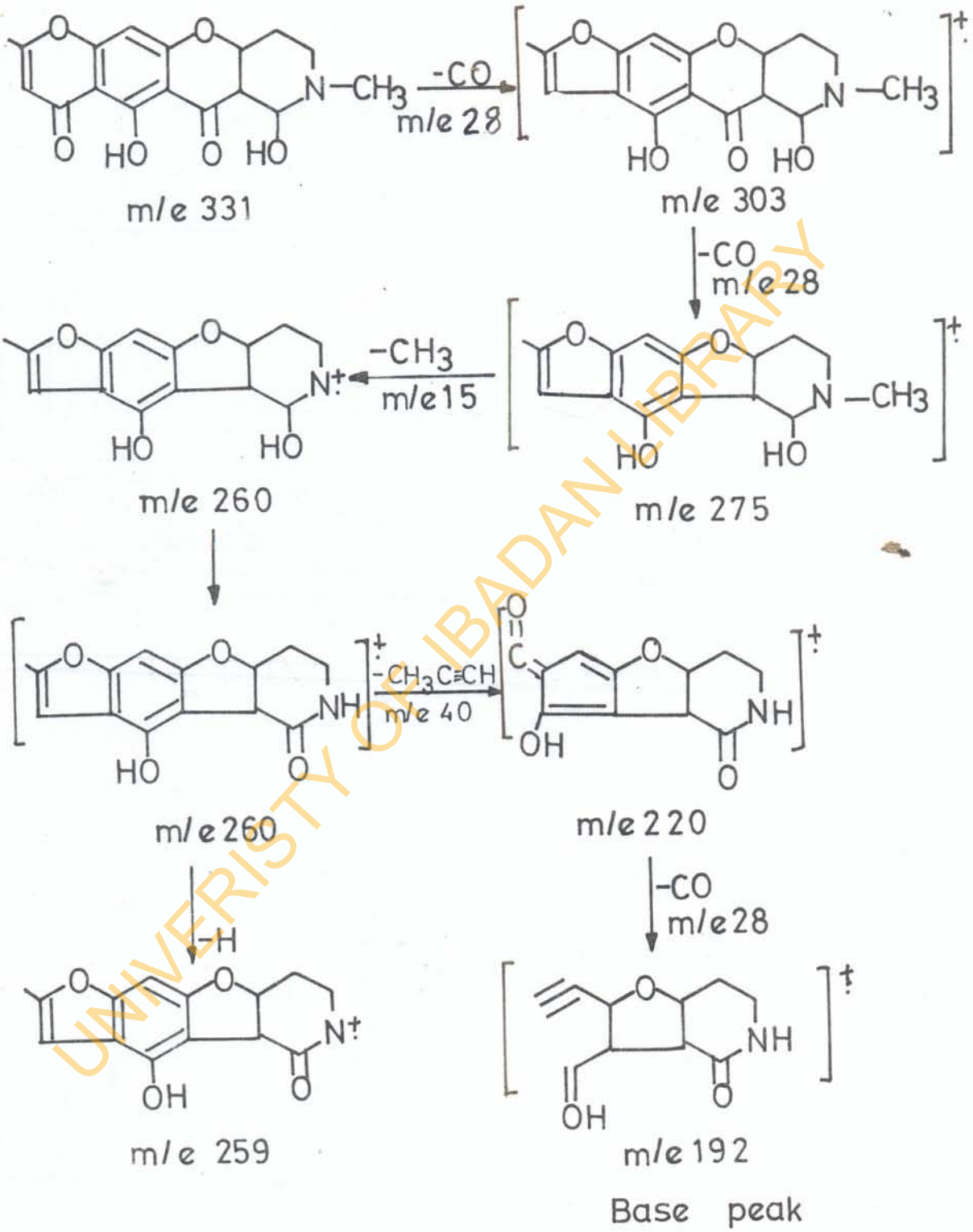
R = CH_3 ; m/e 217
R = H ; m/e 203

R = CH_3 ; m/e 189
R = H ; m/e 175

Scheme 18a



Scheme 18b



Scheme 18c

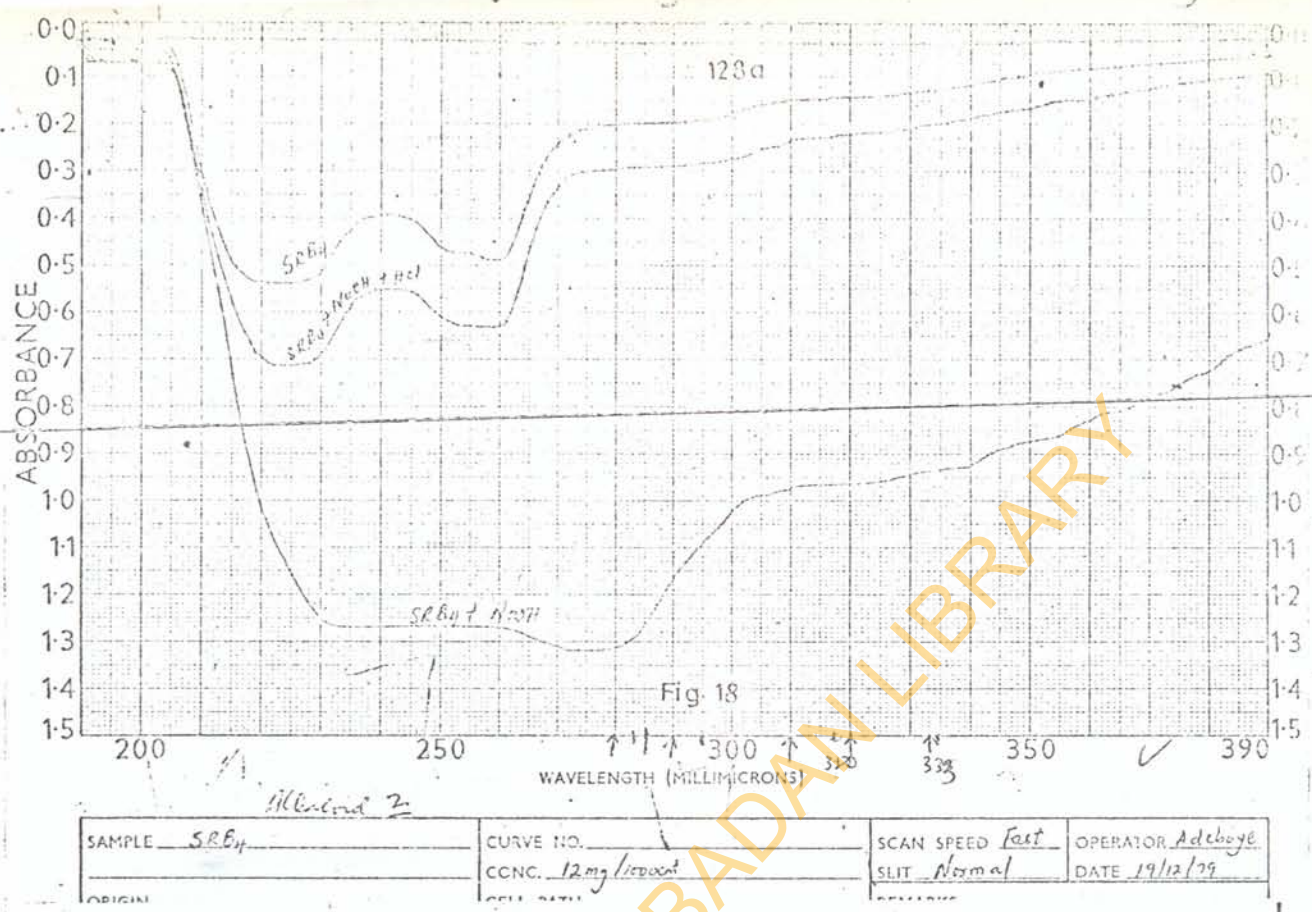


Fig. 18

SAMPLE SRBH	CURVE NO.	SCAN SPEED Fast	OPERATOR Adeboye
	CONC. 12 mg/100cm³	SLIT Normal	DATE 19/12/79
ORIGIN			

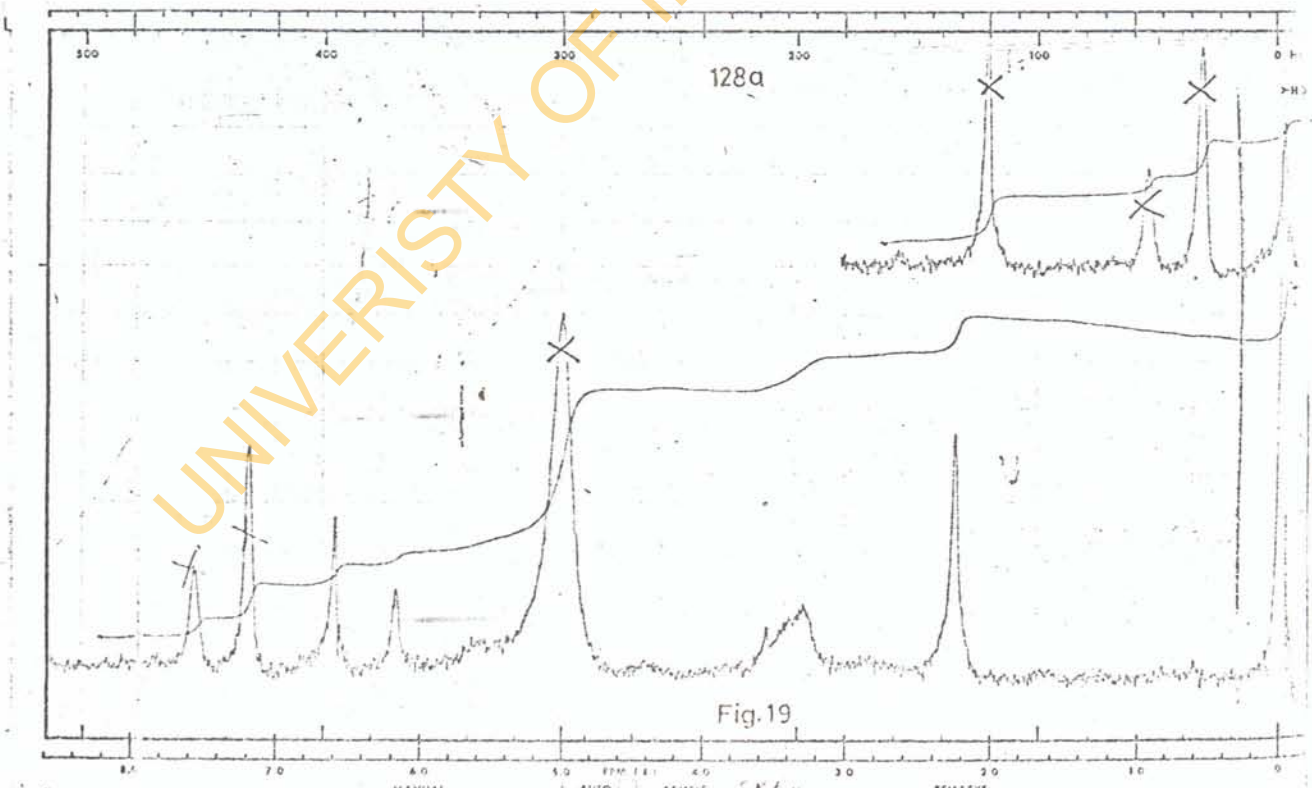


Fig. 19

MANUAL AUTO SAMPLE: SRBH REMARKS:

SWEEP OFFSET (Hz): 0 SWEEP TIME (SEC): (250)

SPECTRUM AMPLITUDE: 1.0 SWEEP WIDTH (Hz): (500)

INTEGRAL AMPLITUDE: 1.0 FILTER: (2)

SPINNING RATE (RPS): 50 RF POWER LEVEL: (.05) SOLVENT: C₆D₅N

X - absorption by solvent

DATE: 19/12/79 OPERATOR: 40 MHz NMR SPECTRUM NO.

retained in the diacetate prepared by treating SRB_4 with a mixture of pyridine and acetic anhydride (where no amide was formed) and in the dimethylether. A weak band appeared at 1710cm^{-1}, while the characteristic chromone carbonyl absorption were observed at 1650cm^{-1}, 1620cm^{-1}(>C=<) ether linkages showed bands at 1165cm^{-1} and 1090cm^{-1}. The substituted aromatic rings showed the characteristic absorption bands at 1580cm^{-1}, 845cm^{-1} and 720cm^{-1}.

The UV spectrum (FIG. 18) taken in methanol was characteristic of the chromone series,[52] showing absorption maxima at λ_{max} (log ε); 220 (4.13), 225 (4.12), 253 (3.86), 260 (3.88), 280 (3.88, sh), 290 (3.85), 310 (3.95, sh), 320 (3.96) and 333nm (3.97, sh). The NMR spectrum of SRB_4 was not studied in isolation but was compared with that of SRB_3 taken in the same solvent and that of 5,7-dihydroxy-2-methylchromone. Since SRB_4 was not soluble in deuterochloroform, the NMR spectrum (FIG. 19) was taken in deuteropyridine. A proton signal was observed as a broad singlet at δ6.20 which was similar to that at the 3-position of the γ-pyrone ring of noreugenin 97. In addition, a three proton singlet which was assigned to the methyl group at the 2-position of γ-pyrone appeared

at δ2.30 and a two proton signal showed up as a singlet at δ6.66.

Initially, it was difficult to assign the latter two protons correctly, but when the NMR spectrum was compared with that of SRB_3 taken in the same solvent, the two proton singlet in the NMR spectrum of SRB_3 was identical with that earlier observed in the NMR spectrum of SRB_4. The NMR spectrum of SRB_3 taken in deuterochloroform confirmed that there was only one aromatic proton which occured as a singlet at δ6.30 and an additional one proton doublet at δ5.70. It was then concluded that in deuteropyridine the proton absorption at δ5.70 shifted to δ6.66. This argument was further supported by the observation on the NMR spectrum of the dimethylether of SRB_4, where an aromatic proton appeared at δ6.35 as one proton singlet and the one proton doublet occured at δ5.68. From the above comparison and deductions, the two proton singlet at δ6.66 in the NMR spectrum of SRB_4 taken in deuteropyridine was assigned to an aromatic proton and a proton at the base of a hydroxyl group which in deuterochloroform appeared at δ5.68 as one proton doublet (J=4Hz). In effect, deuteropyridine shifted the proton

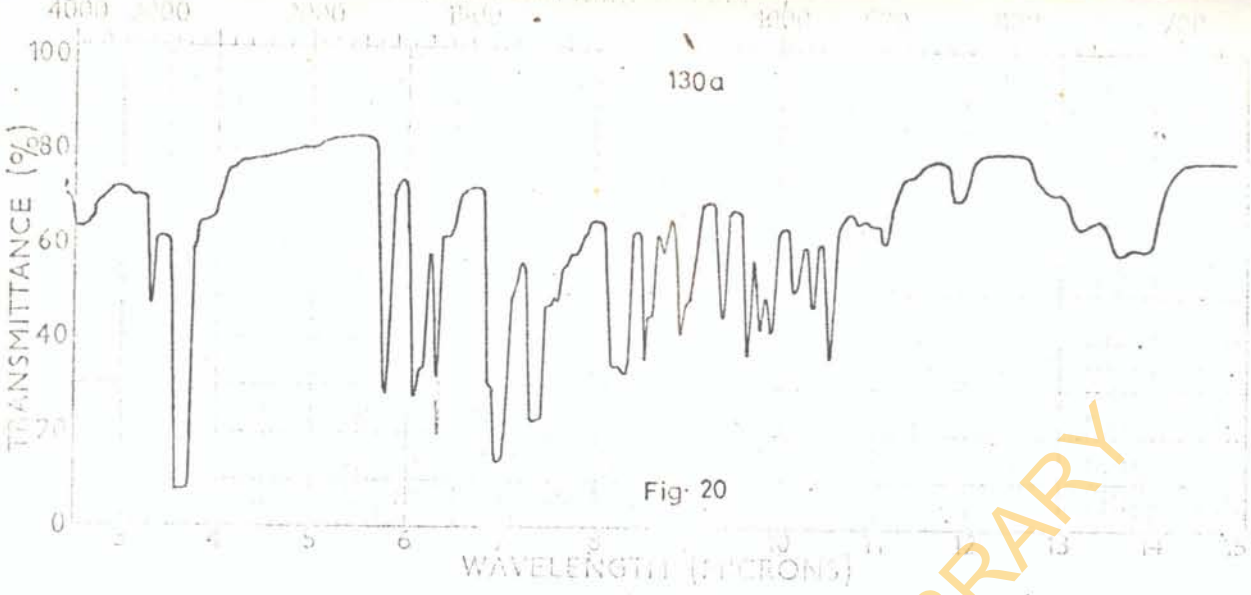


Fig. 20

SAMPLE Acetylated SRBy ACBy

M.P.

ORIGIN

PHASE N

SOLVENT

CONC.

CELL PATH

REFERENCE

CHART SPEED fast SWEEP Normal

OPERATOR J.O. Atebeje DATE 1/5/78

REMARKS

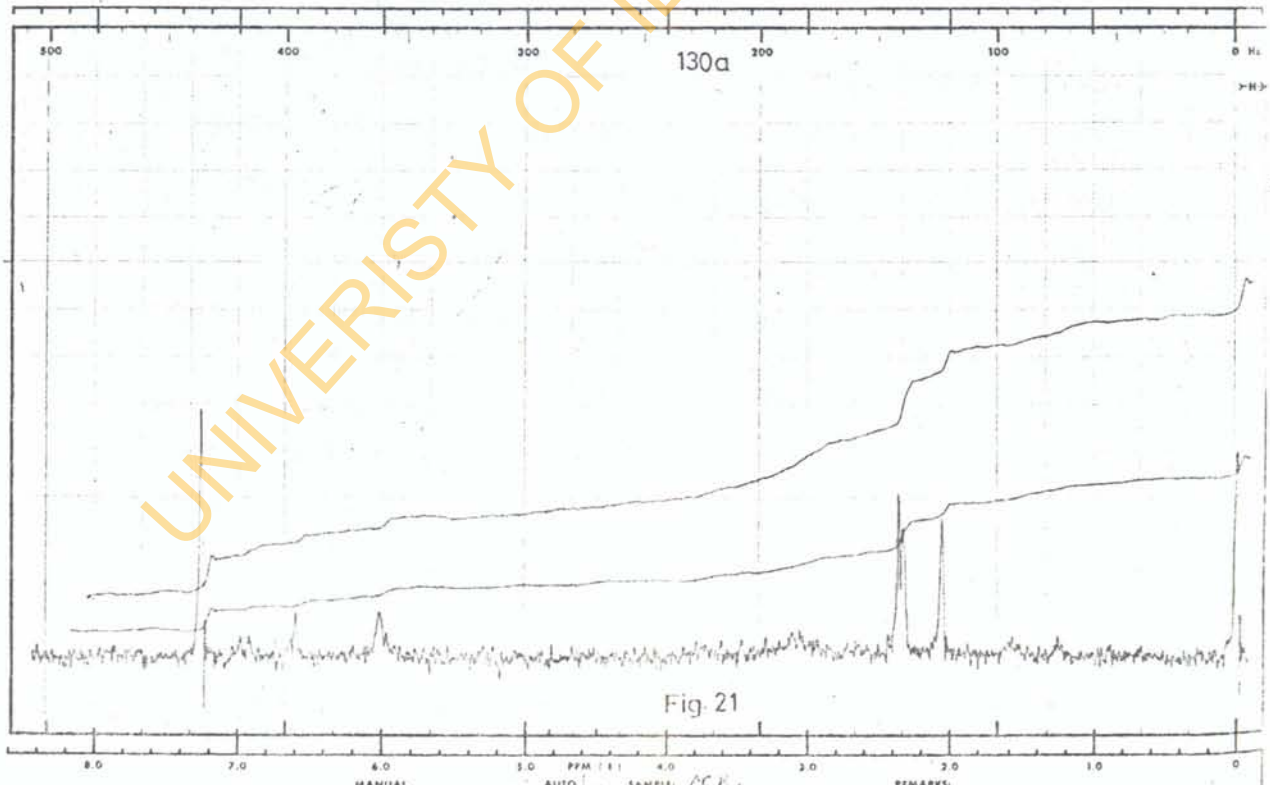


Fig. 21

8.0 7.0 6.0 5.0 PPM (δ) 4.0 3.0 2.0 1.0 0

MANUAL AUTO SAMPLE: ACBy REMARKS:

SWEEP OFFSET (Hz): dummy
SPECTRUM AMPLITUDE: 5.0
INTEGRAL AMPLITUDE: 4.6
SPINNING RATE (RPS):

SWEEP TIME (SEC): (250)
SWEEP WIDTH (Hz): (500)
FILTER: (2)
RF POWER LEVEL: (.05)

SOLVENT: CCl_3

DATE: 24/4/78 OPERATOR:

60 MHz NMR SPECTRUM NO.

at the base of the hydroxyl group downfield and at the same time affected the multiplicity of the signal. The remaining protons could not be accurately estimated from the NMR spectrum because all the acidic protons, e.g. -O-H, and >N-H had been removed by the D_2O in the deuteropyridine.

Acetylation of SRB_4 in a mixture of pyridine and acetic anhydride gave the diacetate (138; $R^1=R^2=Ac, R^3=H$), m.p. 217-219°. The IR spectrum (FIG.20) of the diacetate showed bands at ν_{max} 3150cm^{-1} (>N-H), 1750cm^{-1} (-OAc), 1660 (carbonyl), 1600 (>C=C<, aromatic), 850 and 750 cm^{-1} (substituted aromatic ring). The NMR spectrum (FIG. 21) of the diacetate taken in $CDCl_3$ (sparingly soluble) showed the following proton signals (δppm); 2.05 (3H, s, non-aromatic OAc) 2.32 (3H, s, $-CH_3$ at 2-position of γ-pyrone), 2.36 (3H, s, enol OAc), 3-3.8 (6H, m. CH and CH_2), 6.02 (1H, s, proton at 3-position of γ-pyrone), 6.60 (1H, s, aromatic proton) and 6.93 (1H, d, J = 4Hz, proton at the base of acetoxylgroup).

Treatment of SRB_4 with a mixture of acetic acid and acetic anhydride with a catalytic amount of p-TSA resulted in the acetylation of both the secondary alcohol (>CHOH)

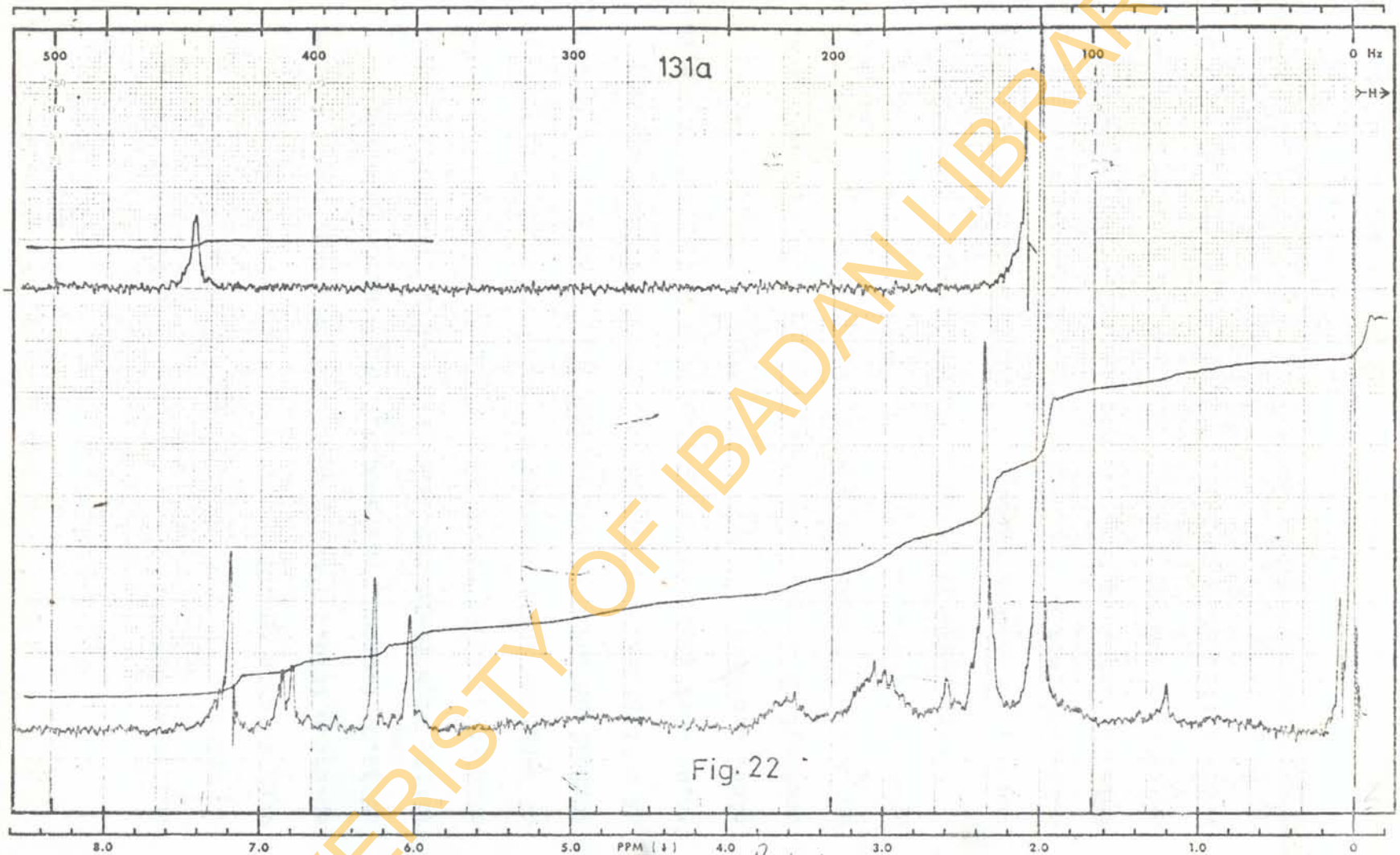


Fig. 22

MANUAL AUTO

SWEEP OFFSET (Hz): 300
SPECTRUM AMPLITUDE: 25
INTEGRAL AMPLITUDE: 3.0
SPINNING RATE (RPS): 40

SWEEP TIME (SEC): 50 (250)
SWEEP WIDTH (Hz): 25 50 100 250 (500)
FILTER: 1 2 3 4 5 6 7 8 (2)
RF POWER LEVEL: 0.05 (.05)

SAMPLE: Product of Acetylation of SRB_4

REMARKS:

SOLVENT: $CDCl_3$

DATE: 1/7/78

OPERATOR:

60 MHz NMR SPECTRUM NO.

and amine (>N-H). The product of acetylation which was an amide of the monoacetate (138; R^1=H, R^2 = R^3 = Ac) was very soluble in $CDCl_3$, unlike the diacetate. The NMR spectrum (FIG.22) taken $CDCl_3$ showed a six proton singlet at δ2.02 (-$OCOCH_3$ and >$NCOCH_3$) which was considered a little high field for an enol acetate. The three-proton singlet assigned to the methyl group at the 2-position of γ-pyrone still appeared at δ2.32 while the six protons (methylene and methine) showed up between δ2.8 and δ3.7 as multiplets. The proton signals at δ6.05, δ6.24 and δ6.84 were assigned to the proton at 3-position of γ-pyrone ring, aromatic proton and the proton at the base of acetoxyl group respectively. The phenolic hydroxyl group which was not acetylated still appeared at δ12.5 as one proton singlet.

This amide of the monoacetate of SRB_4 was not stable and so, on passing through a column of silica gel, it was half-converted to the monoacetate. The evidence for this was obtained from the NMR spectra. The amide was completely converted to the monoacetate during the recrystallization process. The monoacetate (138; R^1 = R^3= H, R^2 = Ac) which had a m.p. 153-155°, was similar in its

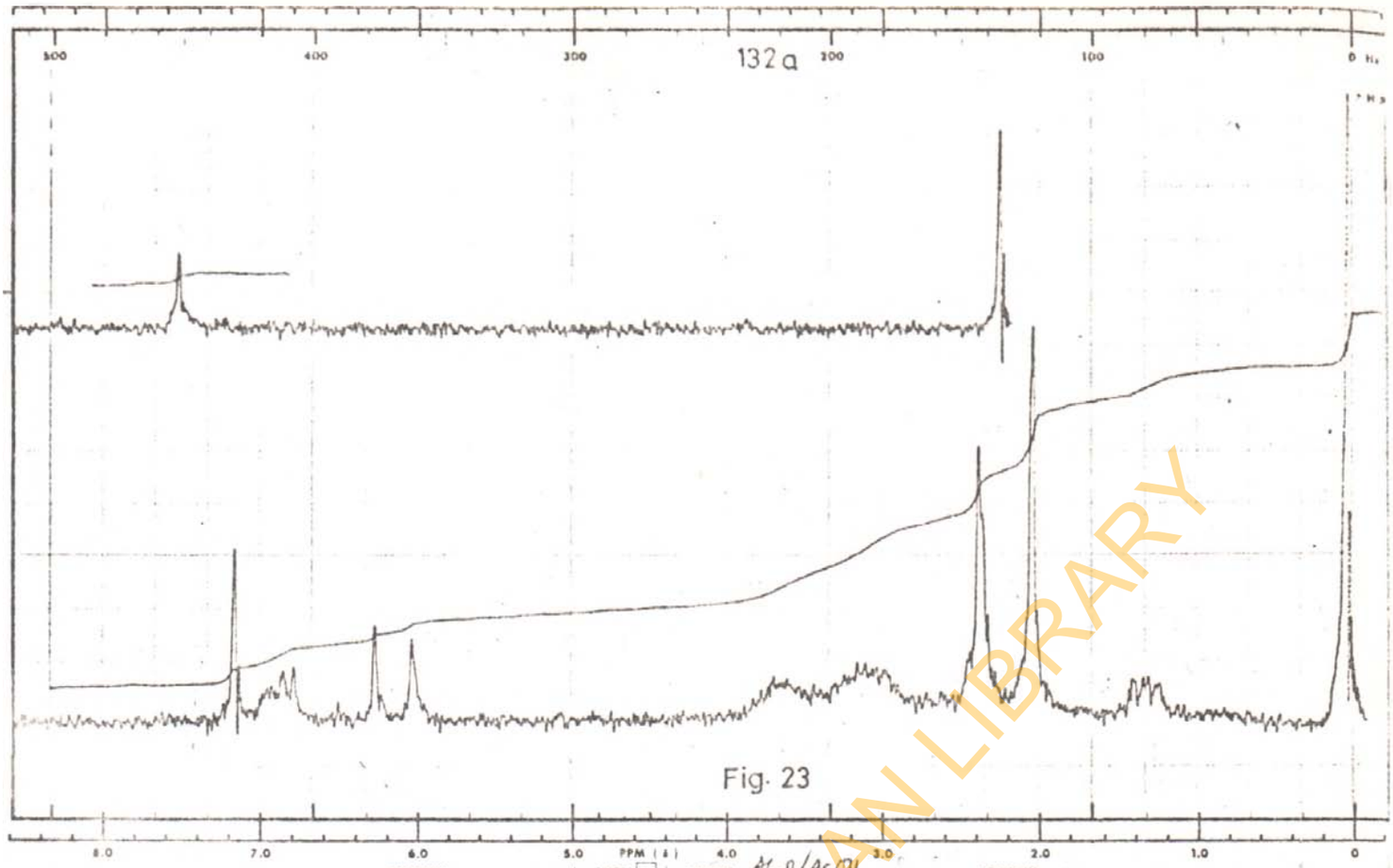


Fig. 23

MANUAL ☐ AUTO ☐ SAMPLE: Ac_2O/AcOH p-TOSH Acetylation of SRB4 REMARKS:

SWEEP OFFSET (Hz): 200
SPECTRUM AMPLITUDE: 32
INTEGRAL AMPLITUDE: 30
SPINNING RATE (RPS): 40
SWEEP TIME (SEC): 50 ☑
SWEEP WIDTH (Hz): 25 50 100 250 500
FILTER: 1 ☑ 3 4 5 6 7 8
RF POWER LEVEL: 0.05
(250)
(500)
(2)
(.05)
SOLVENT: $CDCl_3$

DATE: 6/4/18 OPERATOR: J.O. Adeboye 60 MHz NMR SPECTRUM NO.

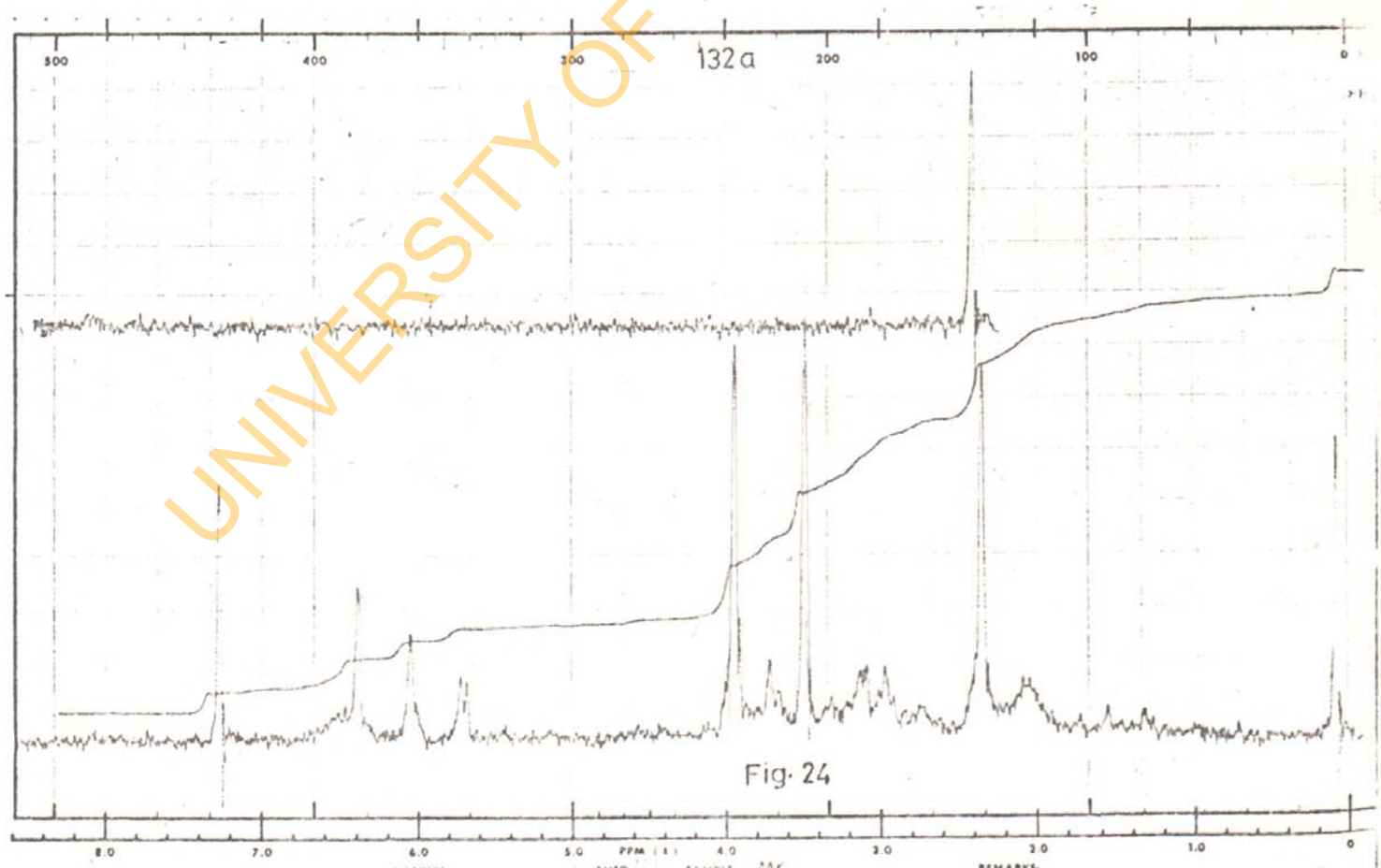


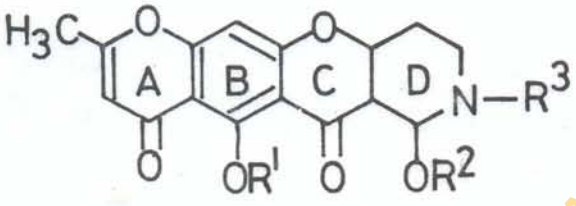
Fig. 24

MANUAL ☐ AUTO ☐ SAMPLE: MF4 REMARKS:

SWEEP OFFSET (Hz): 200
SPECTRUM AMPLITUDE: 40
INTEGRAL AMPLITUDE: 40
SPINNING RATE (RPS): 40
SWEEP TIME (SEC):
SWEEP WIDTH (Hz):
FILTER:
RF POWER LEVEL:
(250)
(500)
(2)
(.05)
SOLVENT: $CDCl_3$

DATE: 24/4/18 OPERATOR: J.O. Adeboye 60 MHz NMR SPECTRUM NO.

NMR spectrum (FIG. 23) to that of the amide except for the peak at δ2.02 which was reduced to a three proton singlet from the six proton singlet observed in the case of the amide.



138 ; $R^1 = R^2 = R^3 = H$

The dimethylether (138; $R^1 = R^2 = CH_3$; $R^3 = H$) which was obtained by treating schumannificine (SRB_4) with a mixture of methyl iodide and silver oxide in chloroform[80] had a m.p. 225-226°. The NMR spectrum (FIG.24) was quite informative and showed a three proton singlet at δ2.30 which was the same methyl group at 2-position of γ-pyrone ring, the methylene and methine protons appeared as six proton multiplets between δ3.0 and δ3.80. The two three proton singlets at δ3.40 and δ3.90 were assigned to the non-aromatic and aromatic

DEHYDROSCHUMANNIFICINE

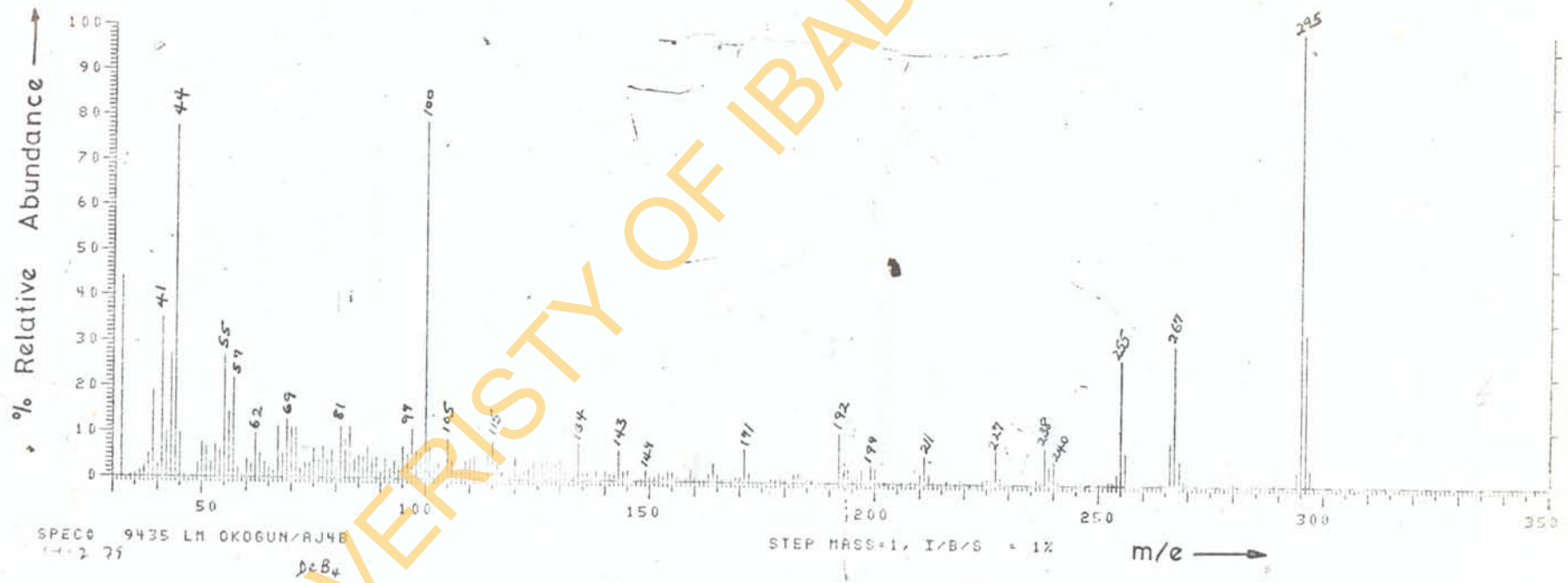


Fig. 25

methoxyl groups respectively, while the proton at the base of the methoxyl group showed up at δ5.68 (J=4Hz) as one proton doublet. The presence of the methoxyl group has no effect on the chemical shift of the base proton, but in the case of the diacetate, amide and the monoacetate, the same proton was shifted downfield to δ6.93 and δ6.84 respectively. This served to prove the relationship between the base proton and the secondary alcohol. The proton at the 3-position of γ-pyrone and the aromatic proton appeared at δ6.01 and δ6.35 respectively in the dimethylether of SRB_4. The NMR spectrum of the dimethylether of SRB_4 suggested a total of 19 protons from the integration which was in agreement with the expected dimethylether of a compound with the molecular formula, $C_{16}H_{15}NO_6$.

SRB_4 was dehydrogenated by a method reported by Ainsworth[86]. Refluxing a mixture of SRB_4 and palladium on carbon in nitrobenzene gave a product which had a m.p. 294--296°. The mass spectrum (FIG. 25) gave the molecular ion, M^+, as 295 which appeared to agree with the molecular ion of a compound with structure 142. Prominent among the peaks obtained from the mass spectrum were: M^+ 295 (100% - base peak), 267 (31%), 255 (28%), 238 (9%), 192 (12%), 172 (8%), 134 (9%), 105 (10%),

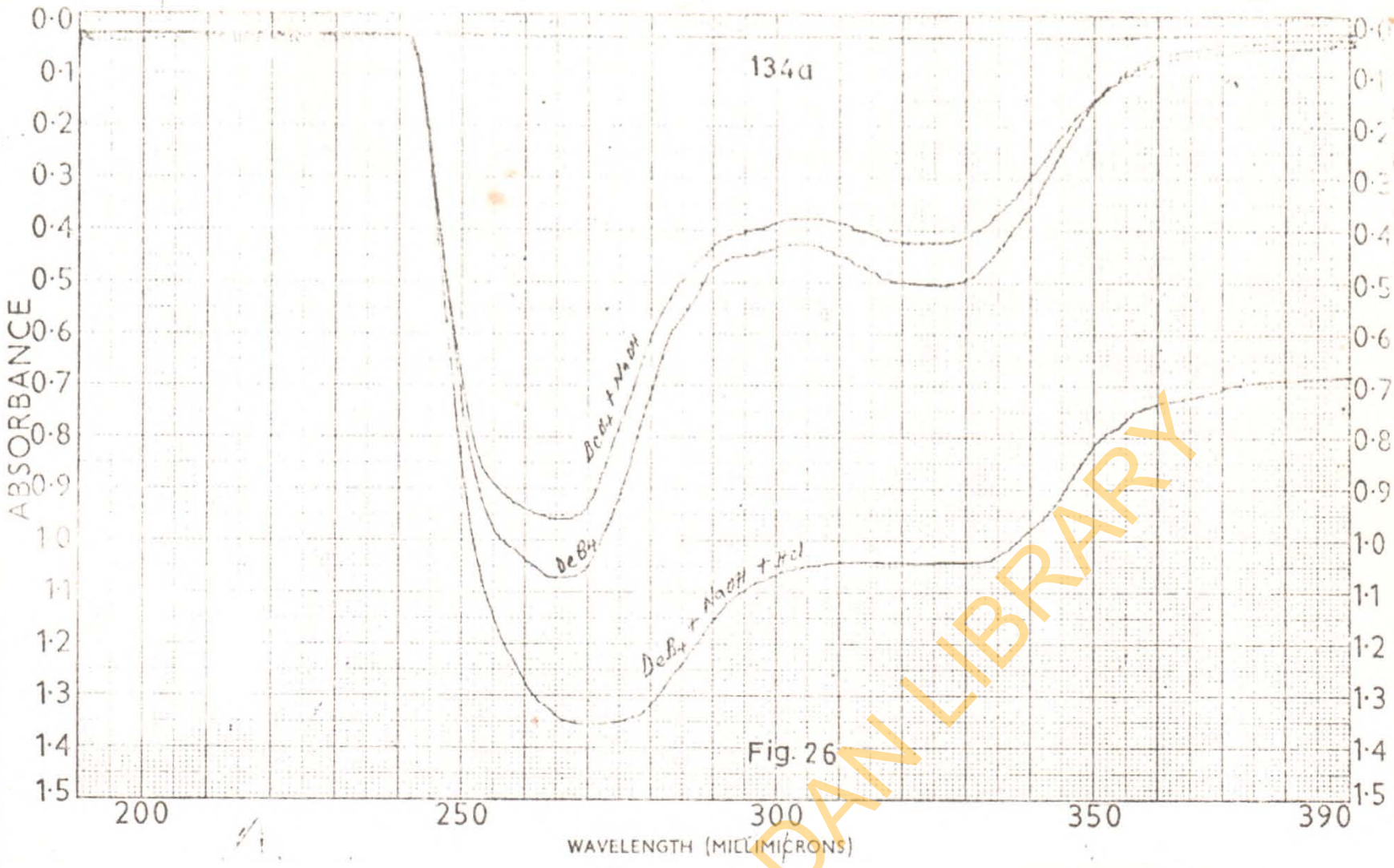


Fig. 26

SAMPLE DeB4	CURVE NO.	SCAN SPEED Fast	OPERATOR Adeboye
	CONC. 12mg/1000cm³	SLIT Normal	DATE 19/12/79
ORIGIN	CELL PATH	REMARKS	
SOLVENT CHCl3	REFERENCE		

PART NO. 202-1511 PERKIN-ELMER LIMITED

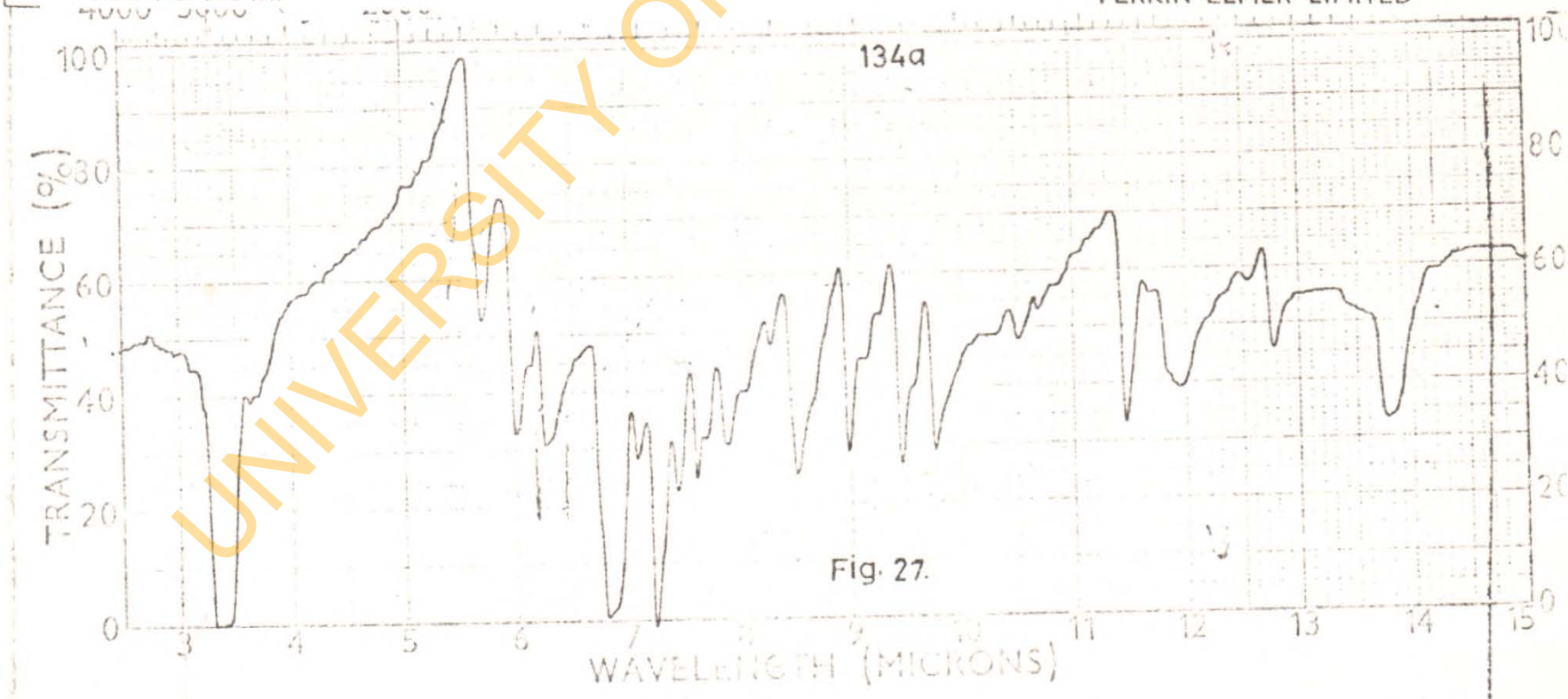


Fig. 27.

SAMPLE DeB4	PHASE N	SCAN SPEED FAST SLIT NORMAL
	SOLVENT	OPERATOR ADEBOYE DATE 26/10/79
	CONC.	REMARKS
	CELL PATH	
ORIGIN	REFERENCE	

PART NO. 202-1511 PERKIN-ELMER LIMITED, BEACONSFIELD, BUCKS

100 (79%), 92 (11%), 83 (12%), and 69 (13%).

The UV spectrum (FIG.26) taken in chloroform (due to insolubility in MeOH and EtOH,) showed the following absorption maxima, λ_{max} (nm) (log ϵ), 258 (4.38), 265 (4.41), 269 (4.41), 321 (4.06) and 328 (4.07). The higher values of the extinction coefficient when compared with the values obtained for 5,7-dihydroxy-2-methylchromone or schumannificine (SRB_4) suggested the introduction of additional conjugation.

What appeared striking in the infrared spectrum (FIG. 27) of the dehydrogenation product was the absorption band at 1740cm^{-1}. This band which seemed to be characteristic carbonyl absorption of aryl ester or lactone was high for aryl ketone or the hydrogen-bonded carbonyl group of the γ-pyrone. It was not easy to explain the high value of the band because the band at 1710cm^{-1} in the starting material, i.e. SRB_4 was too weak and of much lower intensity when compared with that of the dehydrogenation product.

However, it was suggested that, probably the removal of the hydroxyl group during the dehydrogenation which automatically removed one of the hydrogen-bonding affecting the neighbouring carbonyl group and possibly the effect of the nitrogen atom in the ring, might have been responsible for the shifting of that carbonyl band to $1740cm^{-1}$. The bands at $1740cm^{-1}$ in 142 and $1710cm^{-1}$ in SRB_4 are difficult to explain.

However, according to Williams and Fleming[101], the carbonyl group in lactams, which absorbs at $1745cm^{-1}$ in four-ring lactam can be shifted by $+15cm^{-1}$ because of the presence of an additional double bond, as in R-CO-N-C=C or -C=C-CO-N. This is an unusual effect for α,β-unsaturation and it is said to be due to the inductive effect of the -C=C- on the well conjugated -CO-N system. Although in compound 142, the nitrogen atom is not in the α-position to the carbonyl group, it is well conjugated with the carbonyl group in the system (-CO-C=C-N), since it is in the γ-position. This is a vinylogous relationship. So, probably the same explanation can be adduced for the unusual high value of the carbonyl function in compound 142. In addition, since, the possibility of rearrangement to lactone during the dehydrogenation

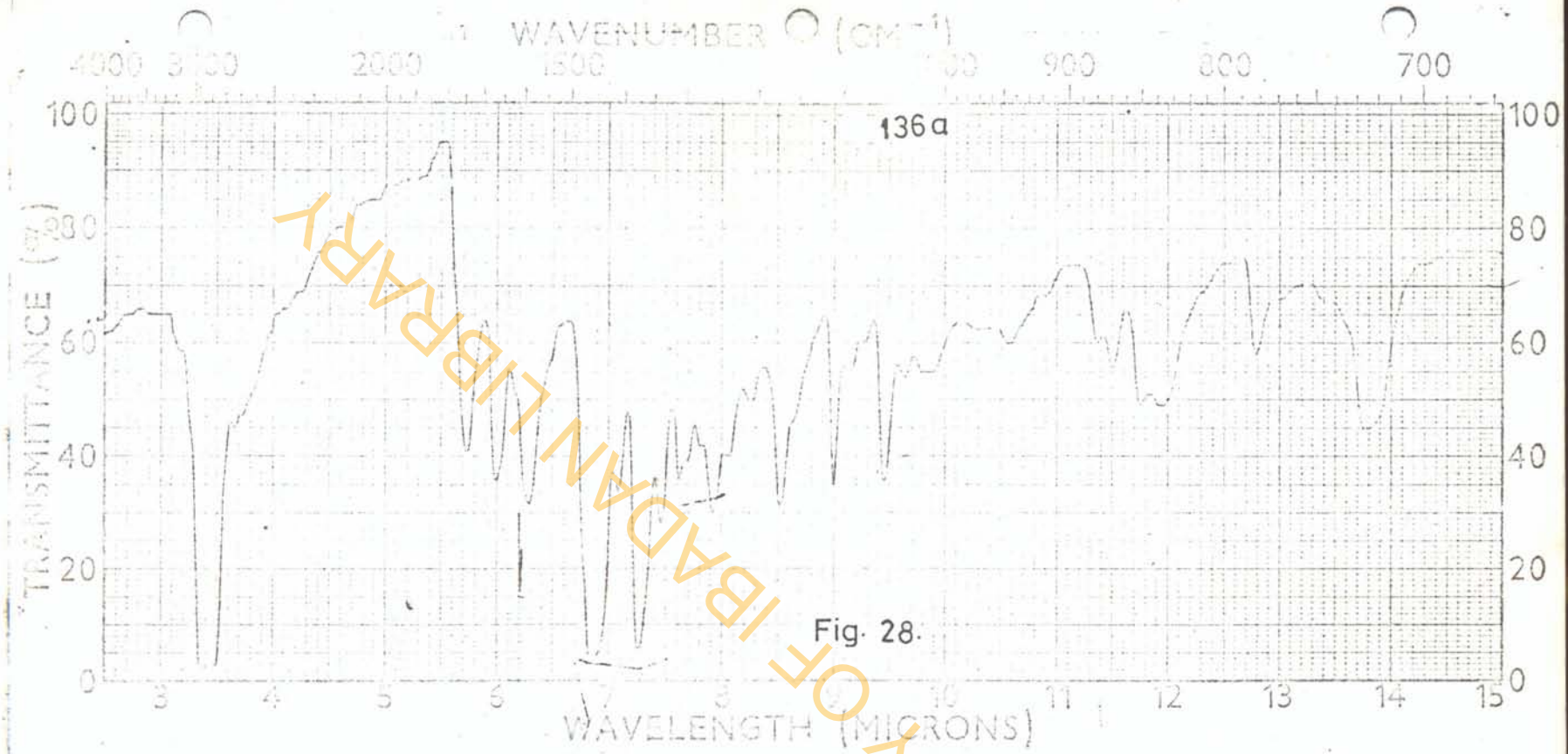
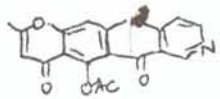


Fig. 28.

SAMPLE Acetylated DeB4



Mpt. 284 - 285° (subl.)

PHASE Nujol

SOLVENT

CONC.

CELL PATH

ORIGIN

REFERENCE

SCAN SPEED Fast SLIT Normal

RUN BY ADEBOYE DATE 11/4/79

NO.

PART NO. 3375 - 1201

PERKIN-ELMER LIMITED, BEACONSFIELD, BUCKS

reaction is ruled out and there is no lactone band in the starting material (SRB_4) then the band at 1740cm^{-1} could not be assigned to a lactone group. The γ-pyrone carbonyl band appeared at 1660cm^{-1} while the aryl ether bands showed up at 1260cm^{-1} and 1165cm^{-1}.

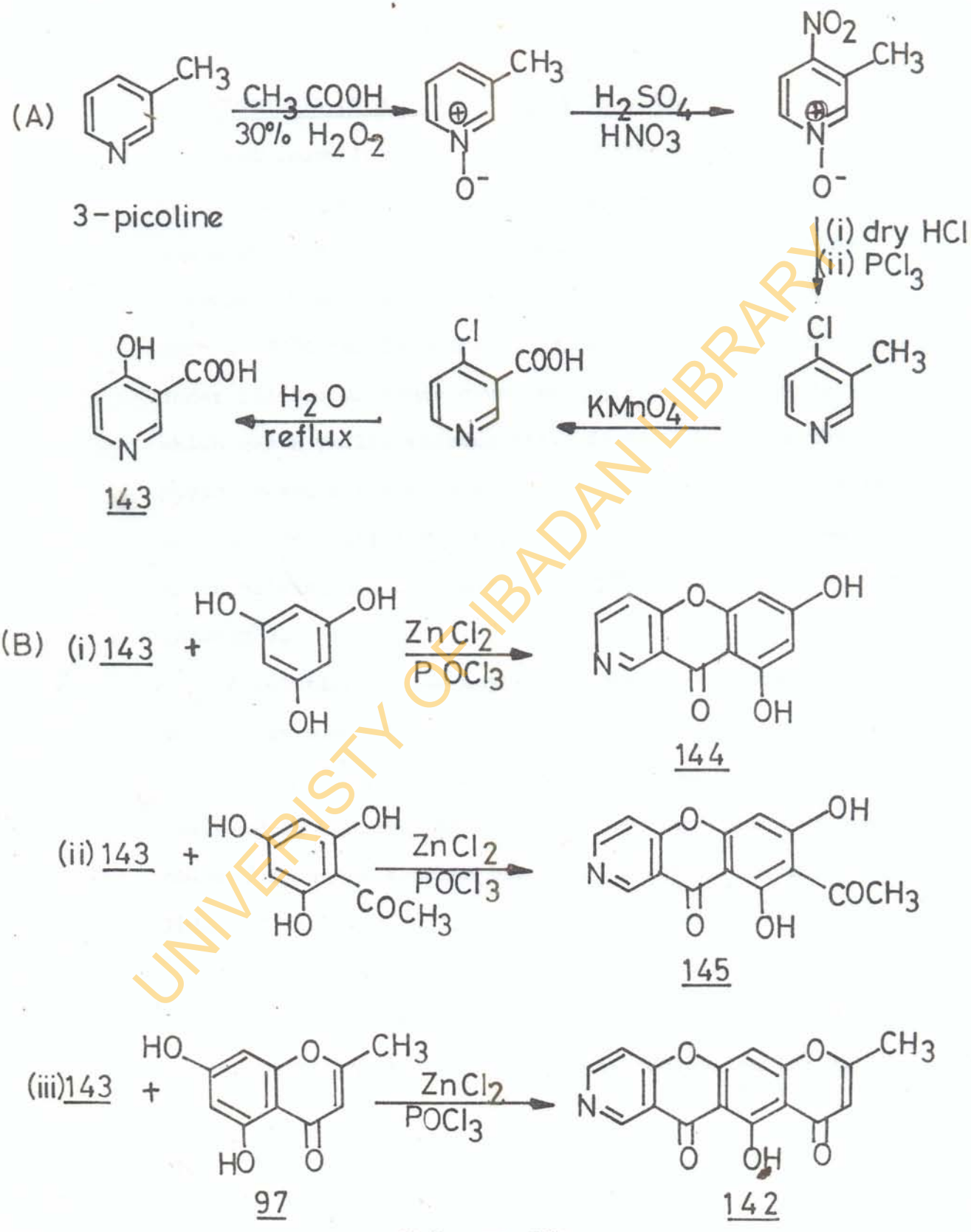
The product of dehydrogenation which proved resistant to acetylation in a mixture of acetic anhydride and pyridine at room temperature was however acetylated when heated in the same mixture at 100°C for 6 hours. This acetate had a m.p. 285-287°(subl.) and the IR spectrum (FIG.28) showed the acetate and carbonyl bands at 1750cm^{-1} and 1670cm^{-1} respectively.

Attempted synthesis of the dehydrogenation product 142

The first synthetic approach involved an acylation reaction between nicotinyl chloride and 5,7-dihydroxy-2-methylchromone 97. Nicotinyl chloride was prepared by treating nicotinic acid with thionyl chloride.[87] Since the resulting nicotinyl chloride was not stable, it was prepared in situ and immediately used for the acylation reaction with 5,7-dihydroxy-2-methylchromone 97. This approach proved unsuccessful.

Attempts were made to acylate 97 and its dimethylether with nicotinic anhydride in the presence of aluminium chloride. The nicotinic anhydride used was prepared by reacting potassium nicotinate with oxalyl chloride in benzene.[88] The method reported by Staudinger[89] was employed in the preparation of oxalyl chloride, though the yield was poor due to the decomposition of oxalyl chloride during fractional distillation. These did not give any positive results.

A well-known synthetic route which leads to the formation of hydroxyxanthones and hydroxybenzophenones[90] involves the treatment of α-hydroxyacids with polyhydroxy compounds in the presence of zinc chloride and phosphorus oxychloride. This, then, meant that α-hydroxy- or 4-hydroxynicotinic acid 143 had to be synthesized. The polyhydroxy compounds such as phloroglucinol, 2,4,6-trihydroxyacetophenone and 5,7-dihydroxy-2-methylchromone were employed in the synthesis. The proposed synthetic route is summarised in scheme 19.



Scheme 19

A. SYNTHESIS OF 4-HYDROXYNICOTINIC ACID.

4-Hydroxynicotinic acid was synthesized by the method reported by Ross[91]. 3-Picoline (3-methylpyridine) was converted into 3-methyl-4-nitropyridine-1-oxide by treating it with aqueous hydrogen peroxide (30%) in acetic acid at 70°C for 24 hours. Evaporation of the solution under reduced pressure gave 3-methylpyridine-1-oxide which was a yellow viscous oil. Treatment of 3-methyl-pyridine-1-oxide with a mixture of concentrated sulphuric acid and concentrated nitric acid afforded 3-methyl-4-nitropyridine-1-oxide, m.p. 135-137° (lit.[91], 137°; lit.[92], 136-138°).

A solution of 3-methyl-4-nitropyridine-1-oxide in chloroform was saturated with dry hydrogen chloride gas at room temperature. Addition of phosphorus trichloride followed by heating under reflux and evaporation of the chloroform under reduced pressure yielded 4-chloropicoline. This was isolated by dissolving the residue left after evaporation in iced water, rendering it strongly basic by adding saturated aqueous sodium carbonate and finally steam-distilling to give pure 4-chloropicoline (d; 1.16g/c.c.).

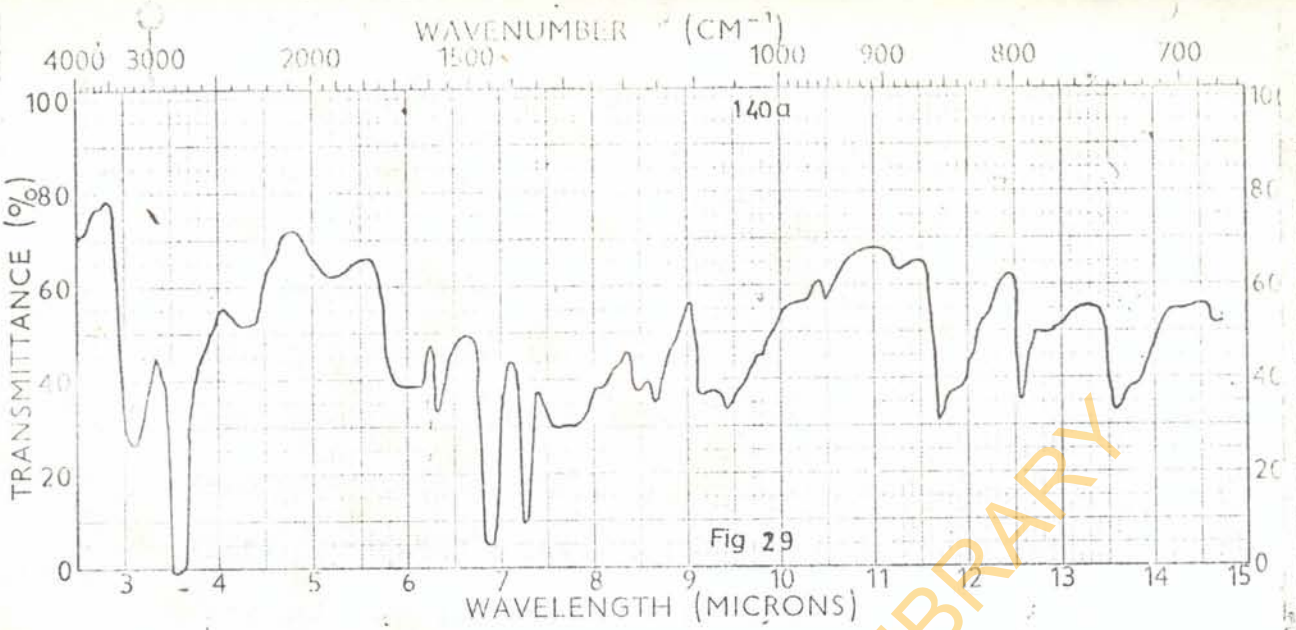
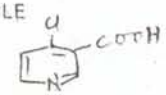


Fig 29

SAMPLE 	PHASE N	SCAN SPEED Fast SLIT Normal
	SOLVENT	OPERATOR Adebaja DATE 25/3/80
	CONC.	REMARKS
	CELL PATH	

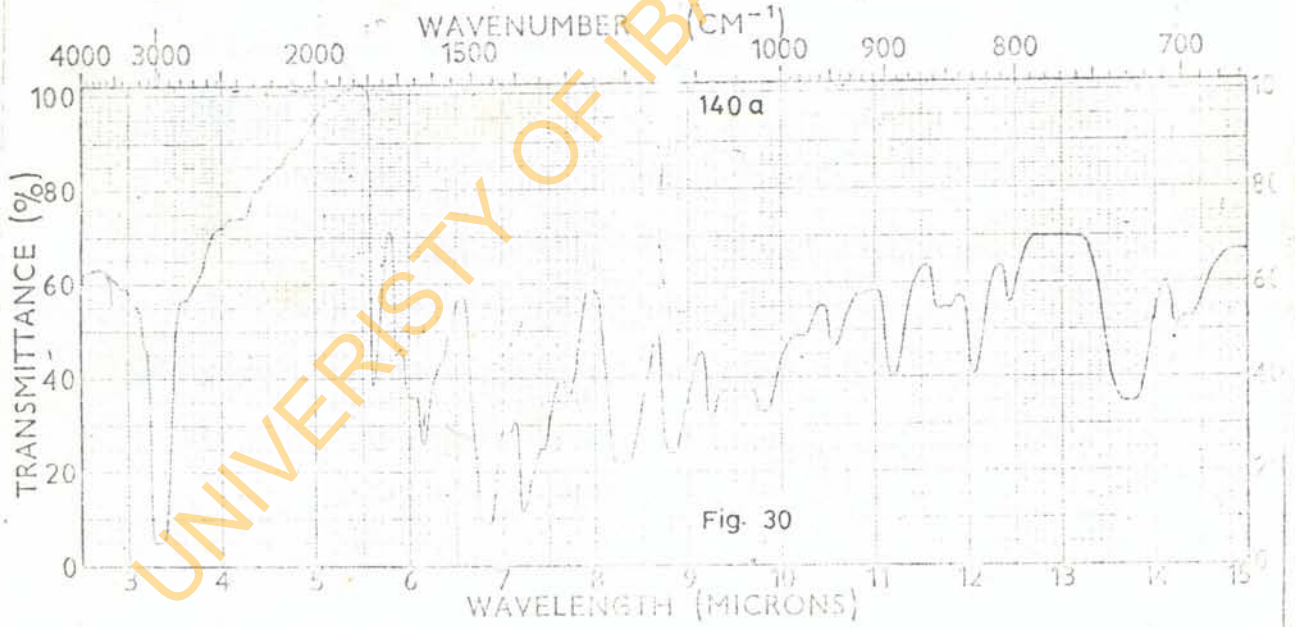


Fig. 30

SAMPLE *m*-Hydroxyphenolic Acid	PHASE	SCAN SPEED SLIT
	SOLVENT	OPERATOR DATE
	CONC.	REMARKS
	CELL PATH	
ORIGIN	REFERENCE	

4-chloropicoline was oxidized to 4-chloronicotinic acid by dispersing it in water and adding potassium permanganate. Heating it for 4 hours at 80-90°C gave 4-chloronicotinic acid m.p. 174-176° (decomp.), (lit.[91], 175-177°; lit.[93], 162-163°, lit.[94], 164°). This acid was isolated by filtering off the precipitated manganese dioxide, concentrating the filtrate and finally adjusting the pH of the concentrated filtrate to 3 with concentrated hydrochloric acid. The infrared spectrum (FIG. 29) showed the hydroxyl group of an acid at 3220cm^{-1} and the carbonyl absorption at 1725cm^{-1} and 1630cm^{-1}.

4-chloronicotinic acid was converted to 4-hydroxynicotinic acid 143 by refluxing it in water for 1 hour. After adjusting the pH of the solution to 4 with sodium hydroxide, evaporation to half bulk and cooling afforded the 4-hydroxynicotinic acid, m.p. 249-250° {lit.[91], m.p. 250°; 260° (decomp)}. The molecular ion, M$^+$, was given as 139 (mass spectrum). In the IR spectrum (FIG. 30) of 4-hydroxynicotinic acid, the hydroxyl and ; the carbonyl bands appeared at 3250cm^{-1}, 3100cm^{-1} and 1750cm^{-1}, 1700cm^{-1} respectively.

B. CONDENSATION REACTIONS.

Attempts were made to react 4-Hdroxynicotinic acid 143 with phloroglucinol, 2,4,6-trihydroxyacetophenone and 5,7-dihydroxy-2-methychromone 97 separately in the presence of freshly fused zinic chloride and phosphorous oxychloride.[90] This was with a view to obtaining 144, and 145 respectively as intermdiates in the synthesis of 142. At the recommended temperature, that is, 60-70°C, there was no reaction in both cases. When the temperature was increased the reaction mixture was charred. Since the same reaction worked with ortho-hydroxybenzoic acid, then the hetero nitrogen had hindered the reaction by its electron donating ability and tendency to form complexes with the lone pair of electrons from nitrogen. It was not surprising then, that when either nicotinic acid or 4-hydroxynicotinic acid was heated with 2,4,6-trihydroxyacetophenone or 5,7-dihydroxy-2-methylchromone at 180°C in the presence of zinc chloride[95], there was no condensation.

Hoesch synthesis[96] was employed in thethird route designed to lead to 146 as an intermediate to 142.

β-Cyanopyridine required for this synthesis was prepared

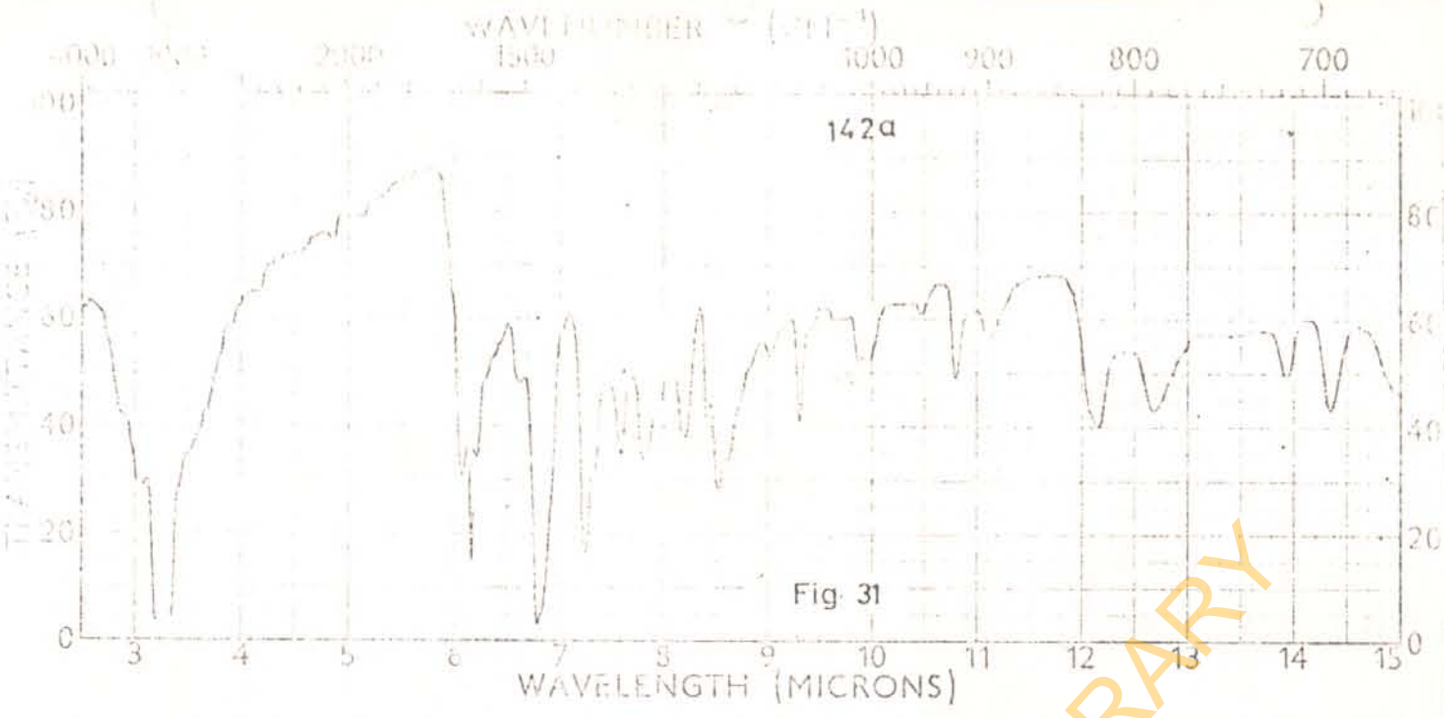
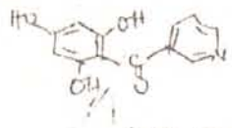


Fig. 31

SAMPLE  M.P: 253-255°C	PHASE Nujol	SCAN SPEED ~~FAST~~ SLIT Normal
	SOLVENT	OPERATOR ABEBOYE DATE 30/4/81
	CONC.	REMARKS
	CELL PATH	
ORIGIN	REFERENCE	

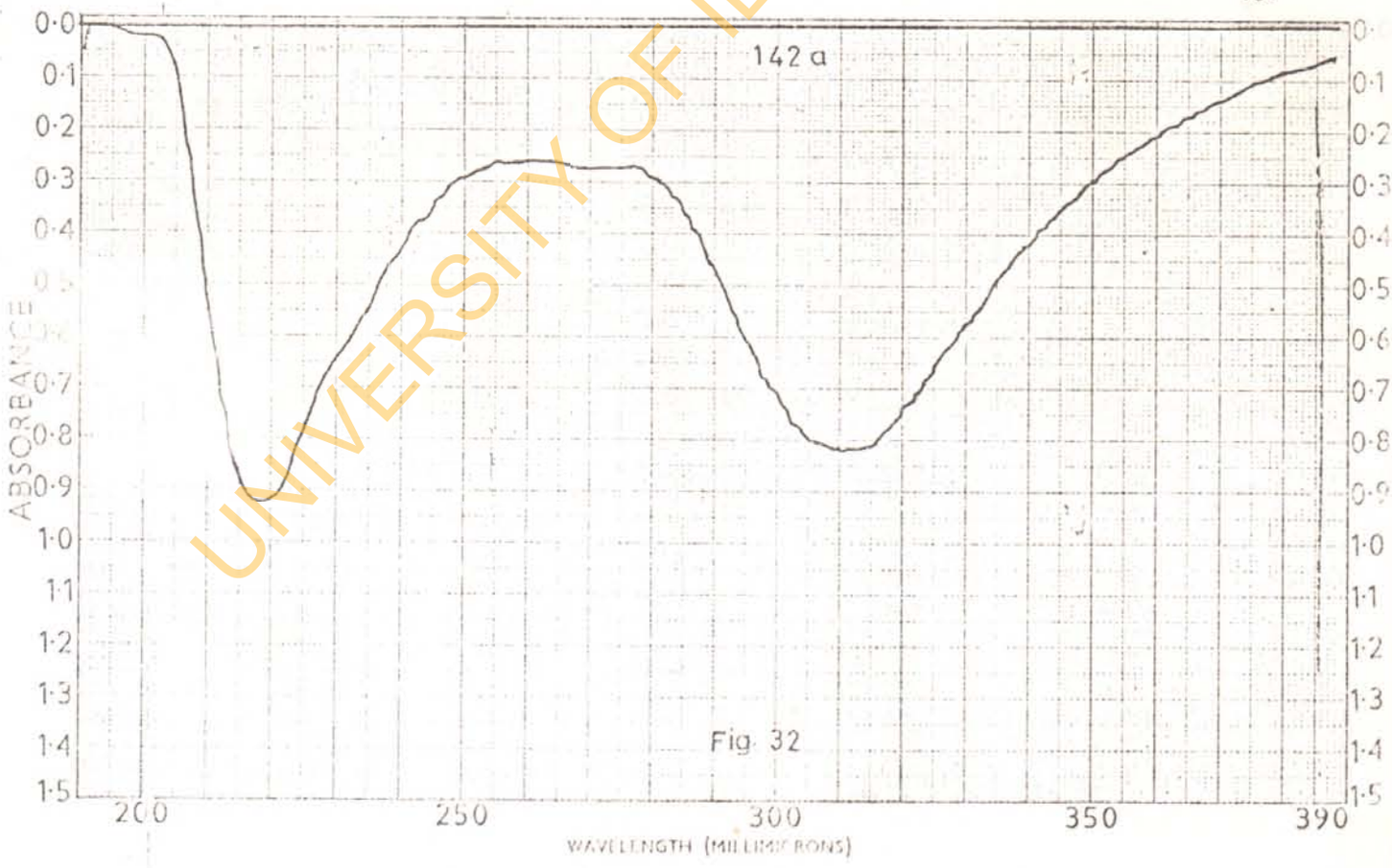


Fig. 32

SAMPLE ABE 56 2,4,6-Tetraphenylpyrylium perchlorate	CURVE NO	SCAN SPEED FAST	OPERATOR ABEBOYE
	CONC. 12 mg/litre	SLIT N	DATE 15-4-81
ORIGIN	CELL PATH	REMARKS	
SOLVENT	REFERENCE		

142b

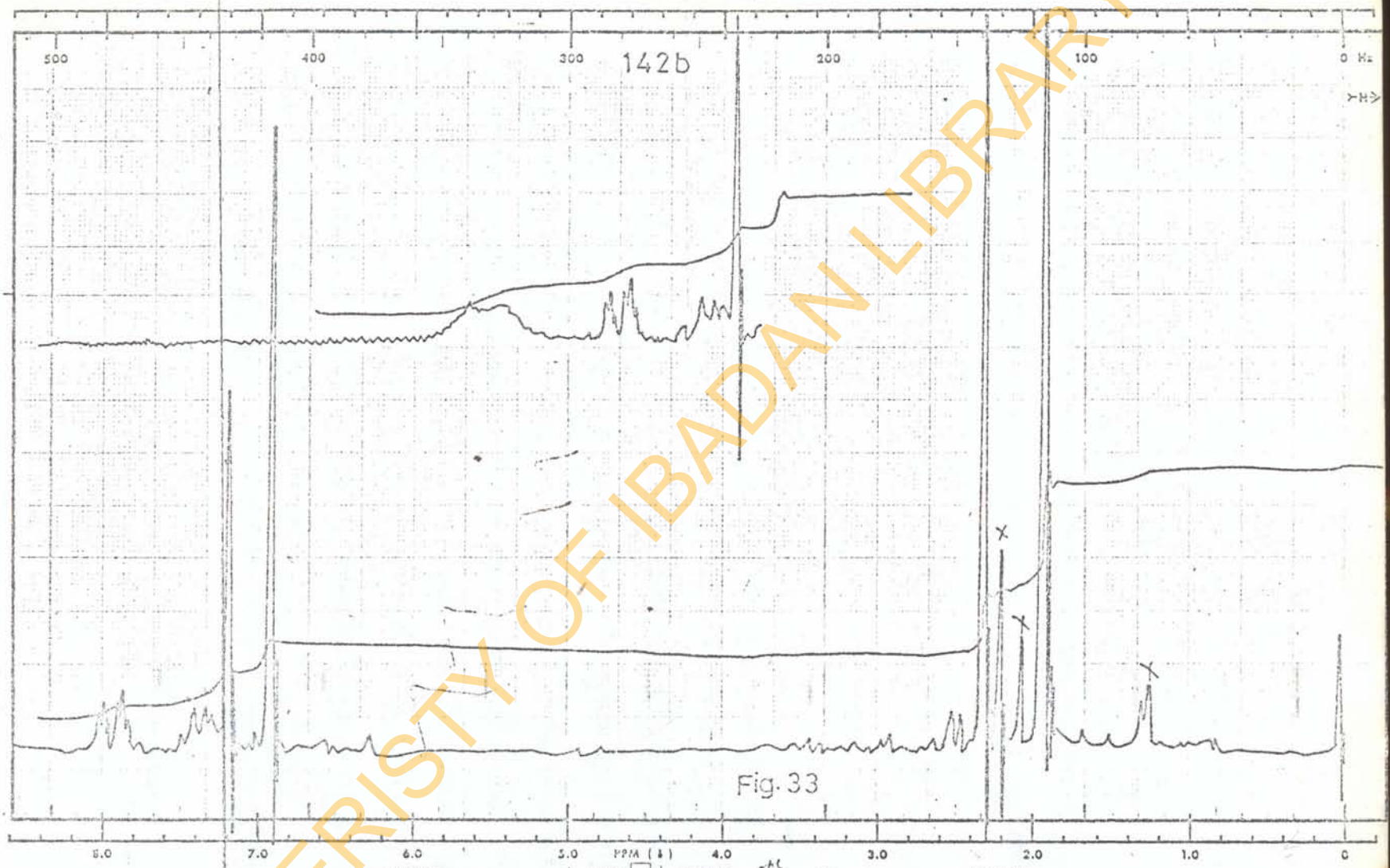


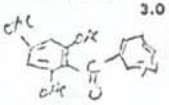
Fig. 33

8.0 7.0 6.0 5.0 PPM (δ) 4.0 3.0 2.0 1.0 0

SWEEP OFFSET (Hz): 200
SPECTRUM AMPLITUDE: 16
INTEGRAL AMPLITUDE: 3.0
SPINNING RATE (RPS): 29

MANUAL
SWEEP TIME (SEC): 50 | 25
SWEEP WIDTH (Hz): 25 | 50 | 100 | 250 | 500
FILTER: 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8
RF POWER LEVEL: 0.05

AUTO ☐
(250)
(500)
(2)
(.05)

SAMPLE: 

SOLVENT: $CDCl_3$

REMARKS:
x - impurity

DATE: 8/4/81 OPERATOR: J. O. ADEBOYE

60 MHz NMR SPECTRUM NO.

by dehydrating nicotinamide with phosphorus pentoxide[97], while the commercial phloroglucinol was used.

Synthesis of 2,4,6-trihydroxynicotinophenone 146

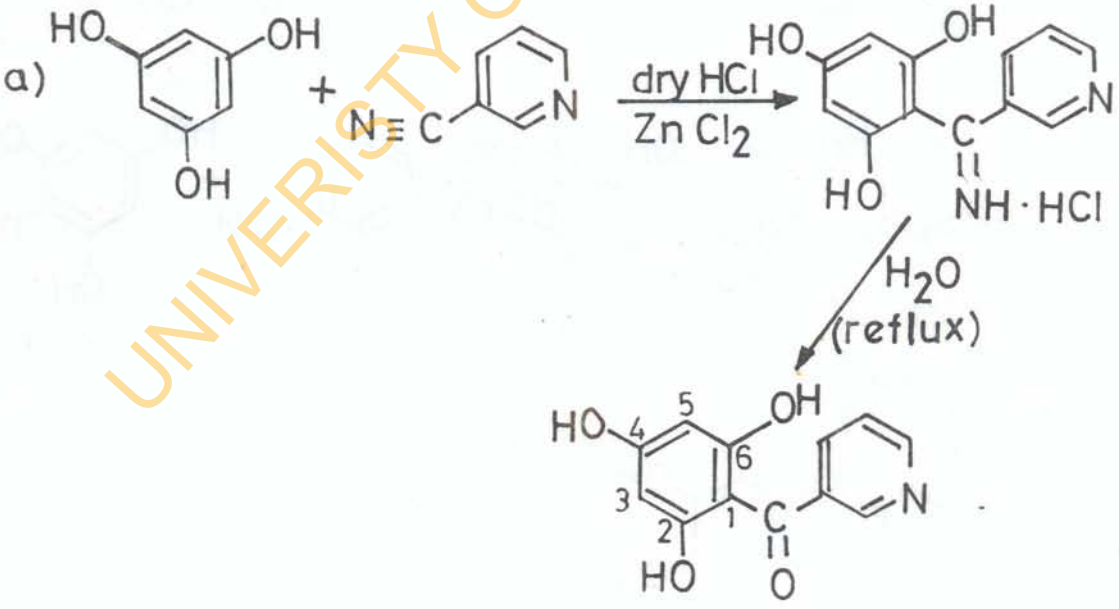
Dry hydrogen chloride gas was passed into a cooled mixture of phloroglucinol, β-cyanopyridine and finely powdered, fused zinc chloride in 1,2-dimethoxyethane (solvent). The reaction flask was left to stand in an ice-chest for 24 hours. After the dry hydrogen chloride has been passed through the solution again, it was kept in a refrigerator for 3 days. The solid precipitate collected was refluxed with water for 2 hours, decolorised with charcoal and filtered hot. This afforded yellow crystals of 2,4,6-trihydroxynicotinophenone, 146 m.p. 253 - 255°C.

The infrared spectrum (FIG. 31) showed the hydroxyl absorptions at 3450cm^{-1}, 3230cm^{-1} and the carbonyl absorption at 1640cm^{-1}. The low carbonyl absorption was due to hydrogen-bonding. The UV spectrum (FIG. 32) taken in methanol has the following absorption maxima, λ_{max} at 217, 221, 305 and 315nm

Compound 146 was not soluble in $CDCl_3$, so the NMR spectrum (FIG. 33) of the triacetate, m.p. 210-211°C, which

was obtained by treating 146 with a mixture of pyridine/Ac_2O gave the following proton signals, δ1.92 (6H, s, two $-OCOCH_3$ at 4- and 6- positions), δ2.30 (3H, s, $-OCOCH_3$ at 2-position), δ6.92 (2H, s, two equivalent aromatic protons at 3- and 5-positions). The four pyridine proton signals occured at δ7.38, δ7.93, δ8.67 and δ8.88.

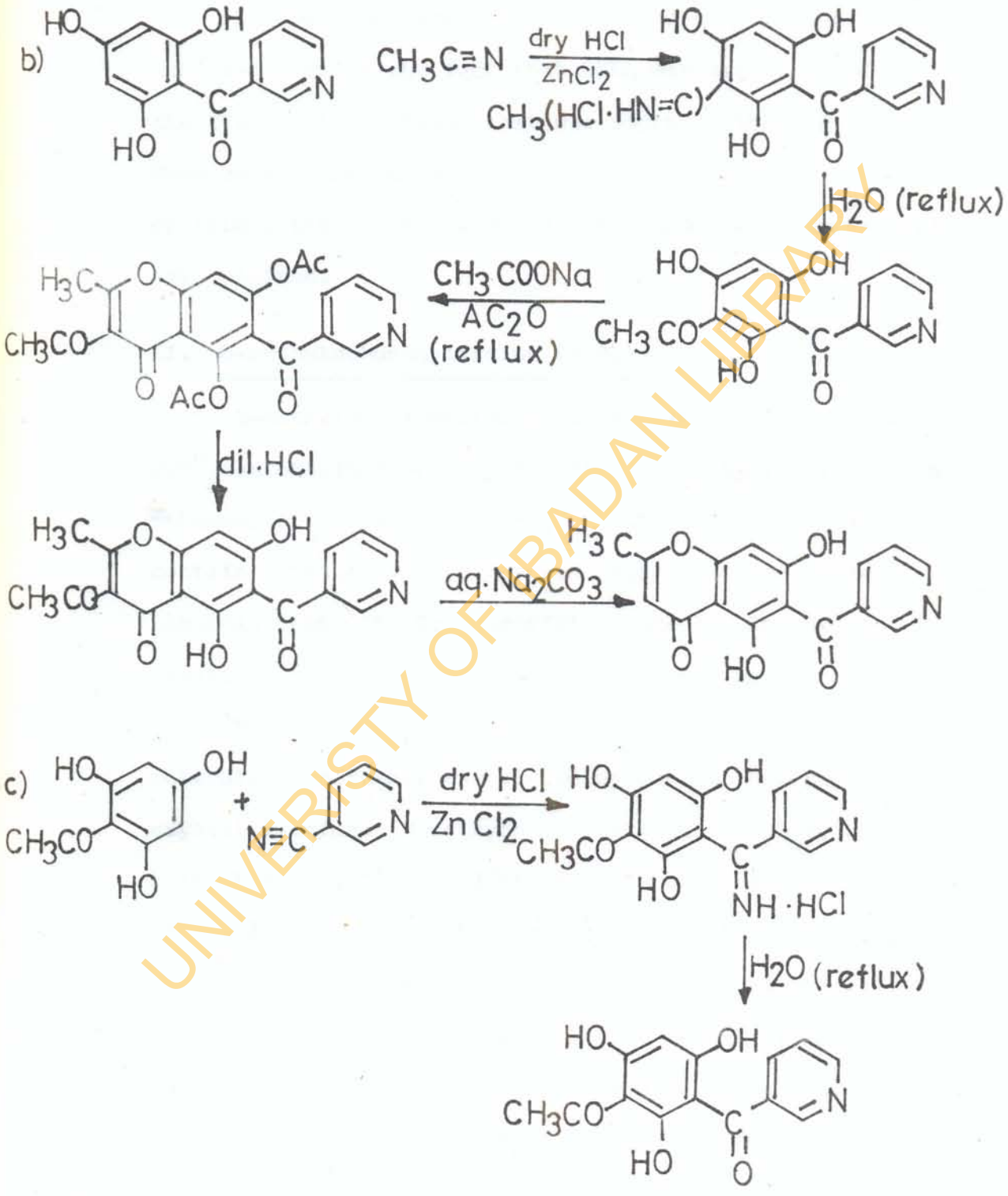
Attempts to introduce the acetyl group into position 3 or 5 in compound 146 by Hoesch synthesis[96] failed. Also, introduction of the nicotinyl group into 2,4,6-trihydroxy-acetophenone by the same method[26] was not possible. The proposed scheme 20(b and c) is shown below.



146

Scheme 20

Scheme 20 contd.



In the above scheme, steps b and c could not be achieved despite the fact that HMPA was used instead of the 1,2-dimethoxyethane that was employed in step a. When both reactions were carried out in HMPA the problem of solubility was removed, but the expected products were not obtained.

II. N-methylschumannificine (SRB_3)

N-methylschumannificine (SRB_3), which had a m.p. 208-209° was obtained pure by fractional crystallization from methanol of a mixture of SRB_3 and SRB_3'. It was shown to contain nitrogen (microanalysis) and gave a positive alkaloid test with Dragendorff's reagent. It gave a positive phenol test with ferric chloride solution.

The elemental analysis gave an indication that one molecule of methanol was picked up during the fractional crystallization. N-methylschumannificine (SRB_3), with molecular ion, M^+ 331 (just 14 units greater than that of SRB_4) was assigned the molecular formula, $C_{17}H_{17}NO_6$.

SRB_3 was found to be closely related to SRB_4. Comparison of the NMR spectra of the two alkaloids taken in deuteropyridine, those of their diacetates and dimethylethers taken in $CDCl_3$ showed that the only

N-METHYLSCHUMANNIFICINE

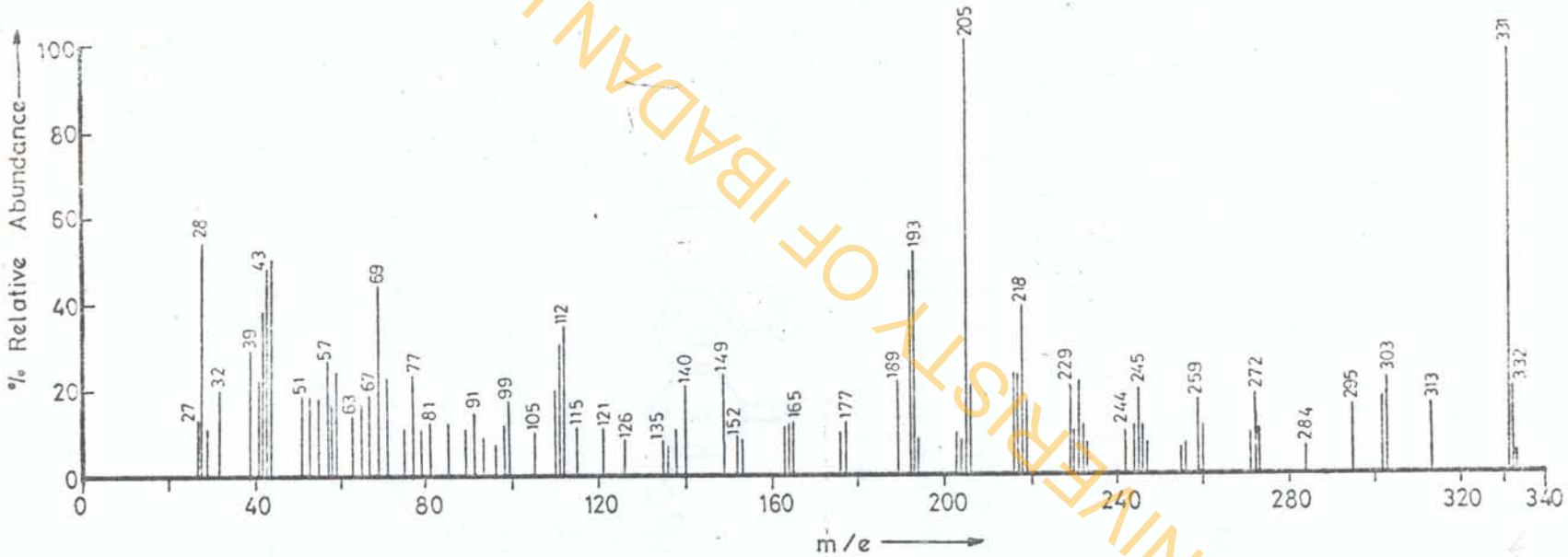


Fig. 34

difference between SRB_3 and SRB_4 was a three proton singlet which appeared at δ2.95 in the NMR spectra of SRB_3 and its derivatives, and was assigned to $>N-CH_3$, but was absent in SRB_4 and its derivatives. SRB_3 was therefore regarded as the N-methylderivative of SRB_4, hence SRB_4 and SRB_3 were named, schumannificine and N-methylschumannificine respectively. SRB_3 was assigned the following structure 147.

147 ; $R^1 = R^2 = H$

Detailed study of the mass spectrum (FIG. 34) of N-methylschumannificine actually showed that the fragmentation pattern was similar to that of SRB_4, thus confirming their close relationship. The proposed fragmentation pattern is shown in schemes 18a, 18b and 18c. Prominent among the peaks observed in the mass spectrum of SRB_3 were: M^+ 331 (97), m/e 313 (16), 303 (22), 219 (22), 218 (39), 206 (20), 205 (100 - base peak), 193 (52), 192 (47), 189 (22), 164 (12), 112 (34) and 69 (44). The percentage relative abundances are shown in the parentheses.

147a

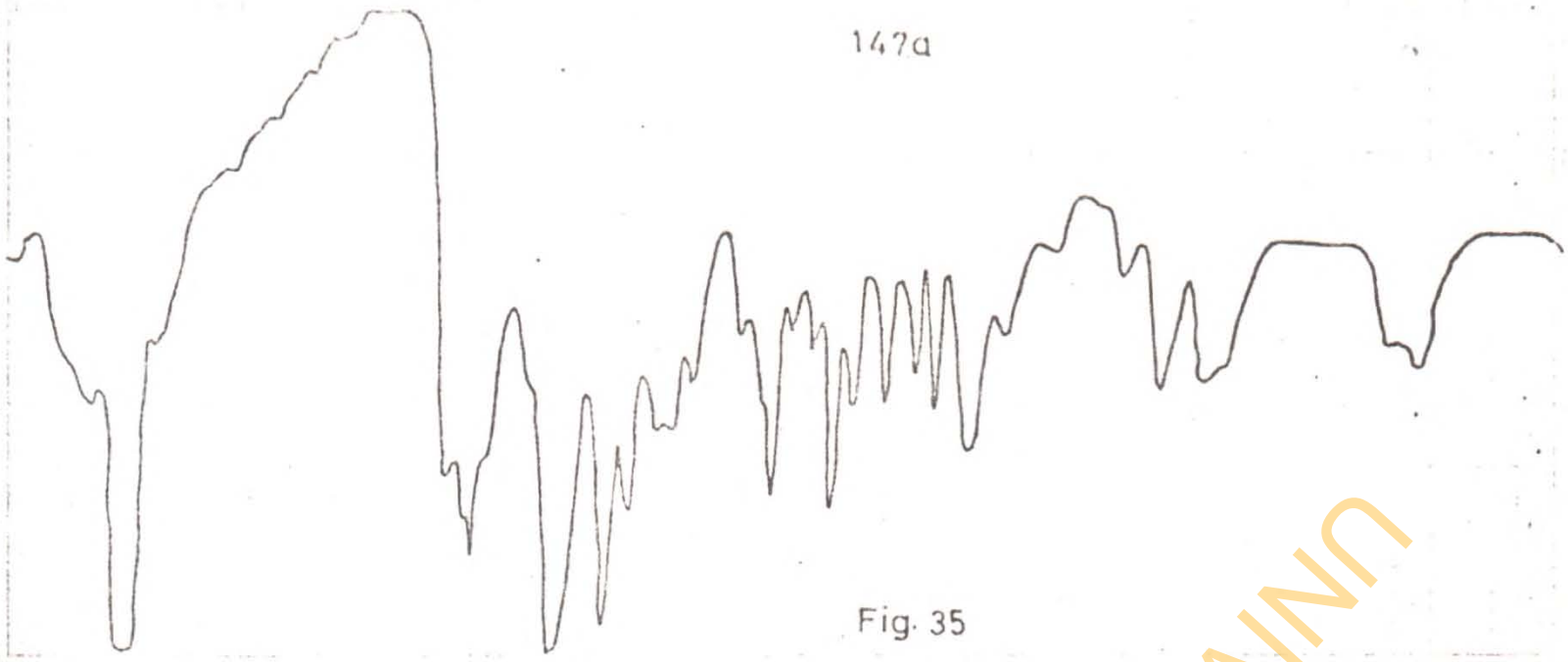


Fig. 35

SAMPLE SRB3 Nujol Fast Normal

Adebaye 17/10/77

UV

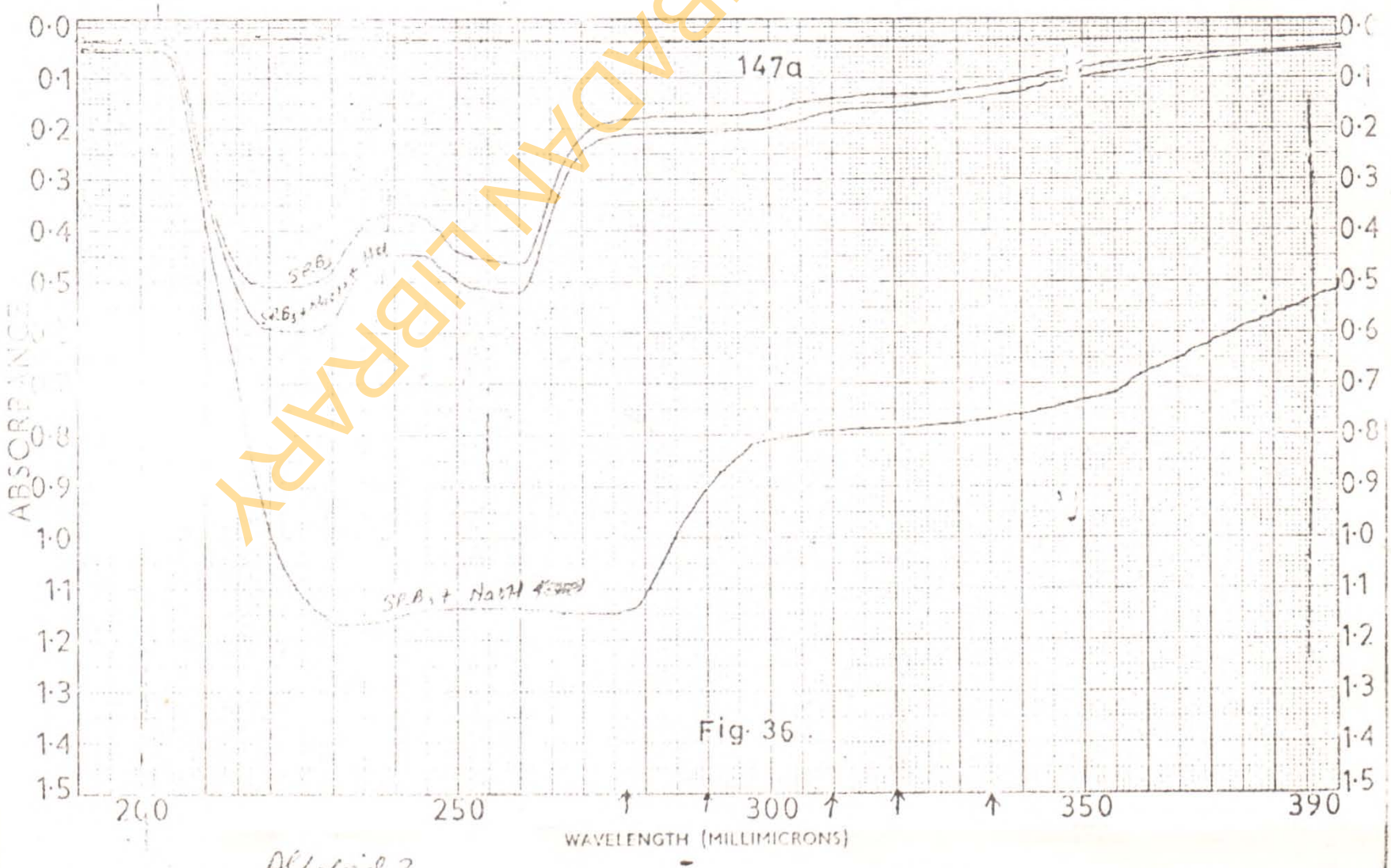


Fig. 36

Alkanil 3

SAMPLE SRB3	CURVE NO.	SCAN SPEED Fast	OPERATOR Adebaye
	CONC. 12 mg/1000cm³	SLIT Normal	DATE 19/12/79
ORIGIN	CELL PATH	REMARKS	
SOLVENT MeOH	REFERENCE		

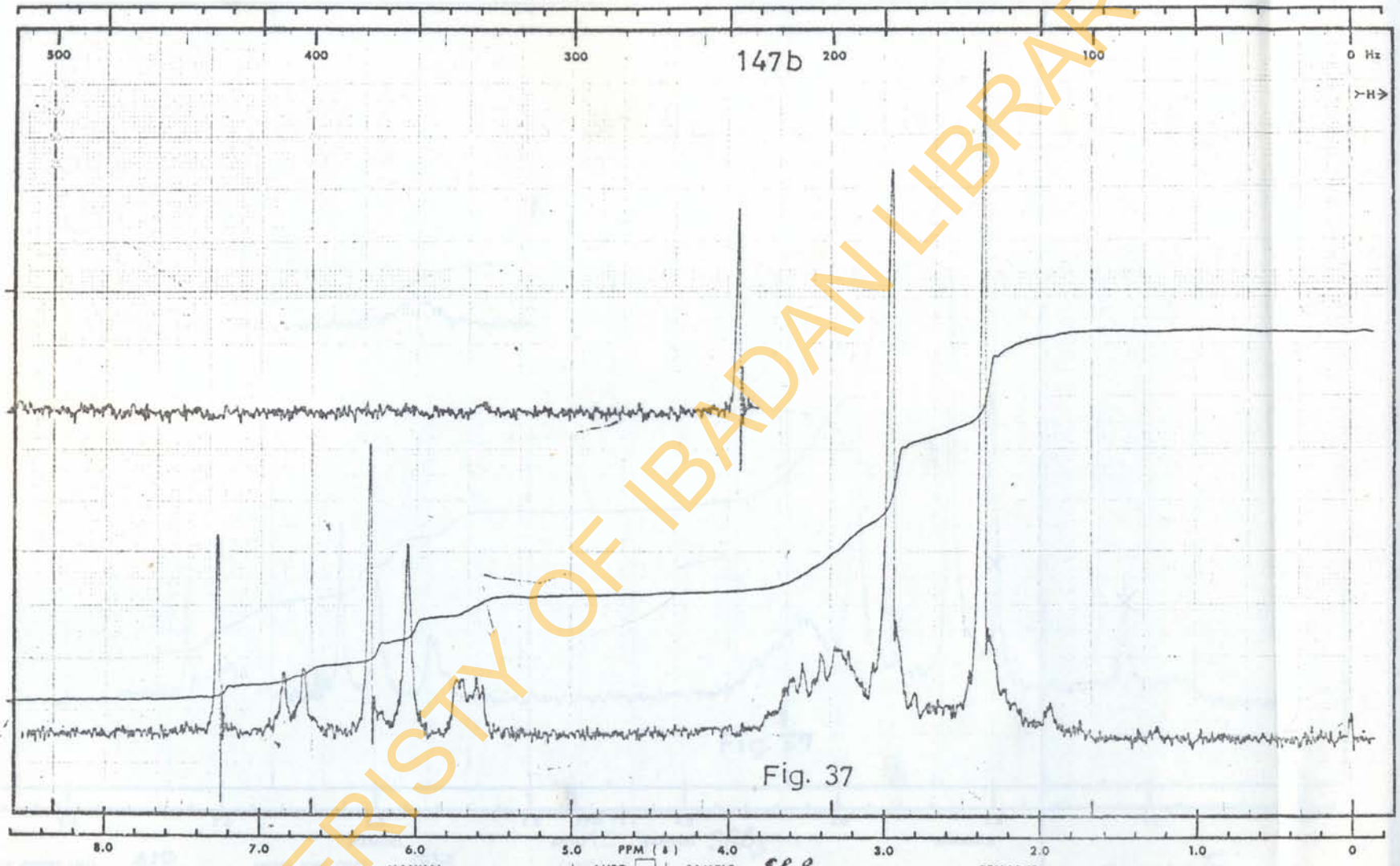


Fig. 37

MANUAL

SWEEP OFFSET (Hz): 100
SPECTRUM AMPLITUDE: 33
INTEGRAL AMPLITUDE: 3.0
SPINNING RATE (RPS): 39

SWEEP TIME (SEC): 50 250
SWEEP WIDTH (Hz): 25 50 100 250 500
FILTER: 1 2 3 4 5 6 7 8
RF POWER LEVEL: 0.05

AUTO ☐
(250)
(500)
(2)
(.05)

SAMPLE: SRB3

REMARKS:

SOLVENT: $CDCl_3$

DATE: 23/6/77

OPERATOR: D.O. Adebaye

60 MHz NMR
SPECTRUM NO.

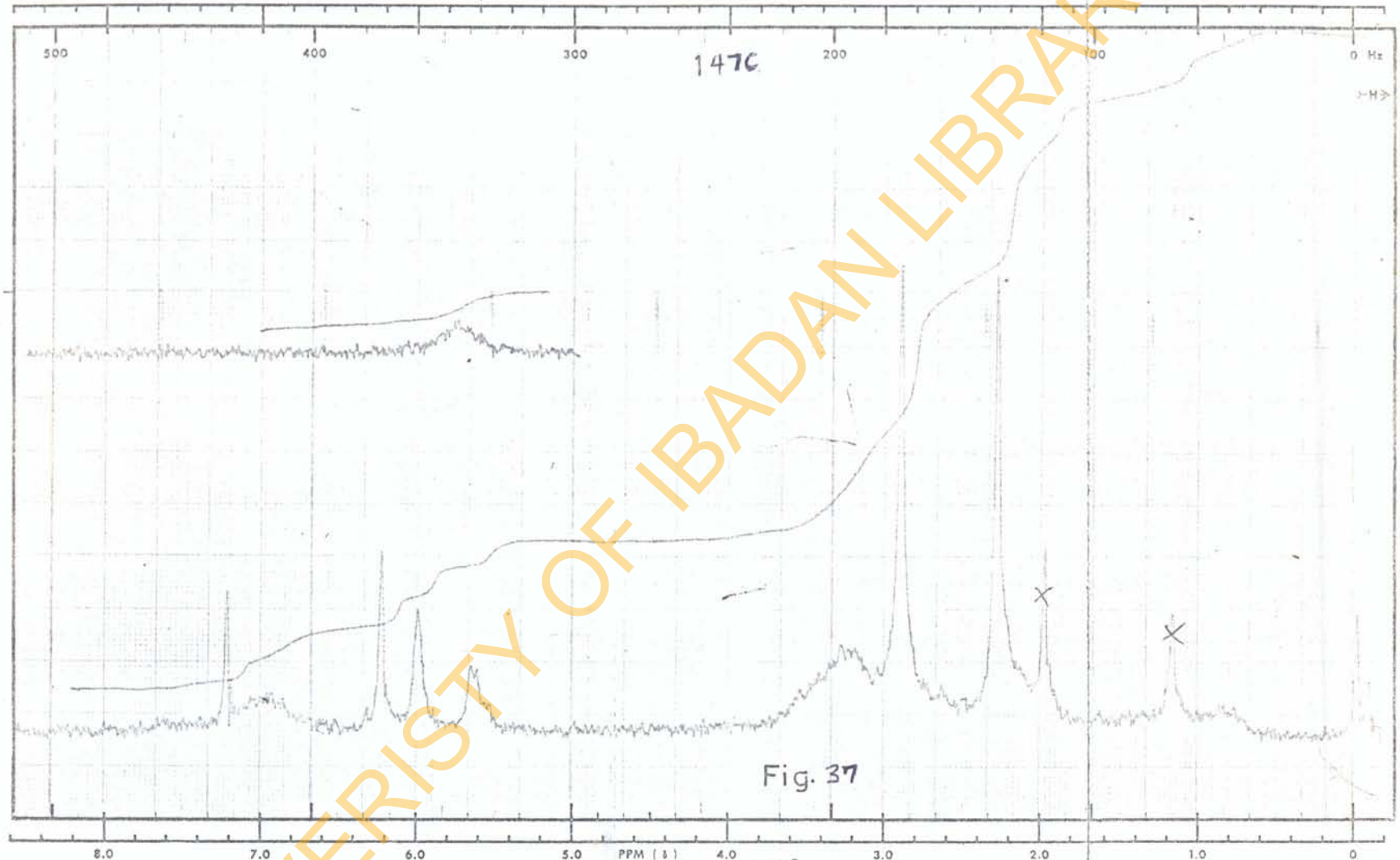


Fig. 37

MANUAL AUTO ☐

SWEEP OFFSET (Hz): 410
SPECTRUM AMPLITUDE: 32
INTEGRAL AMPLITUDE: 4·0
SPINNING RATE (RPS): 40

SWEEP TIME (SEC): 50 (250)
SWEEP WIDTH (Hz): 25 50 100 250 500 (500)
FILTER: 1 2 3 4 5 6 7 8 (2)
RF POWER LEVEL: 0·05 (.05)

SAMPLE: SRB_3

SOLVENT: $CDCl_3$

REMARKS:

X - Impurity

DATE: 15/2/78

OPERATOR: J·O·Adebaye

60 MH: NMR
SPECTRUM NO.

The IR spectrum (FIG. 35) suggested the presence of hydroxyl group (3360~3180cm^{-1}), carbonyl absorption at 1670cm^{-1} and aromatic bands at 1575, 865, 845 and 820cm^{-1}. The UV spectrum (FIG. 36) of the compound taken in methanol showed absorption maxima at λ_{max} 220 (log ϵ = 4.12), 225 (log ϵ = 4.12), 253 (log ϵ = 3.89), 260 (log ϵ = 3.90), 277 (log ϵ = 3.89, sh), 290 (log ϵ = 3.90), 310 (log ϵ = 3.95, sh), 320 (log ϵ = 3.96) and 335nm (log ϵ = 3.98, sh). The above absorption maxima were found to be very similar to the characteristic chromone absorptions.

The NMR spectrum (FIG. 37) taken in deuterochloroform gave the characteristic chromone three proton singlet at δ2.39, which was the methyl group located at 2-position of γ-pyrone ring. The three proton singlet at δ2.95 was assigned to a methyl group attached to a nitrogen atom (>N-CH$_3$) while the six-proton multiplets which showed up between δ3.2 and δ3.6 were assigned to both methylene and methine protons. The proton at the base of hydroxyl group appeared at δ5.7 as one proton doublet (J=4Hz). The proton at the 3-position of γ-pyrone ring and the aromatic proton signals appeared at δ6.06 (1H, s) and

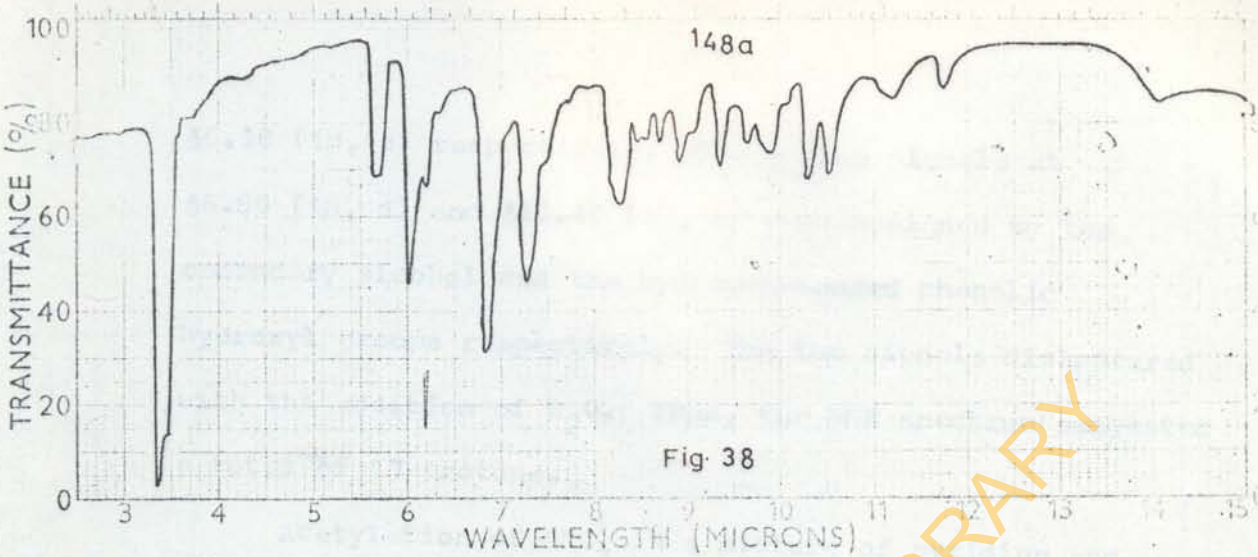


Fig. 38

SAMPLE : AcB_3	PHASE Nujol	SCAN SPEED Fast SLIT Normal
	SOLVENT	OPERATOR J.D Akeroje DATE 25/10/77
	CONC.	REMARKS
	CELL PATH	
ORIGIN	REFERENCE	

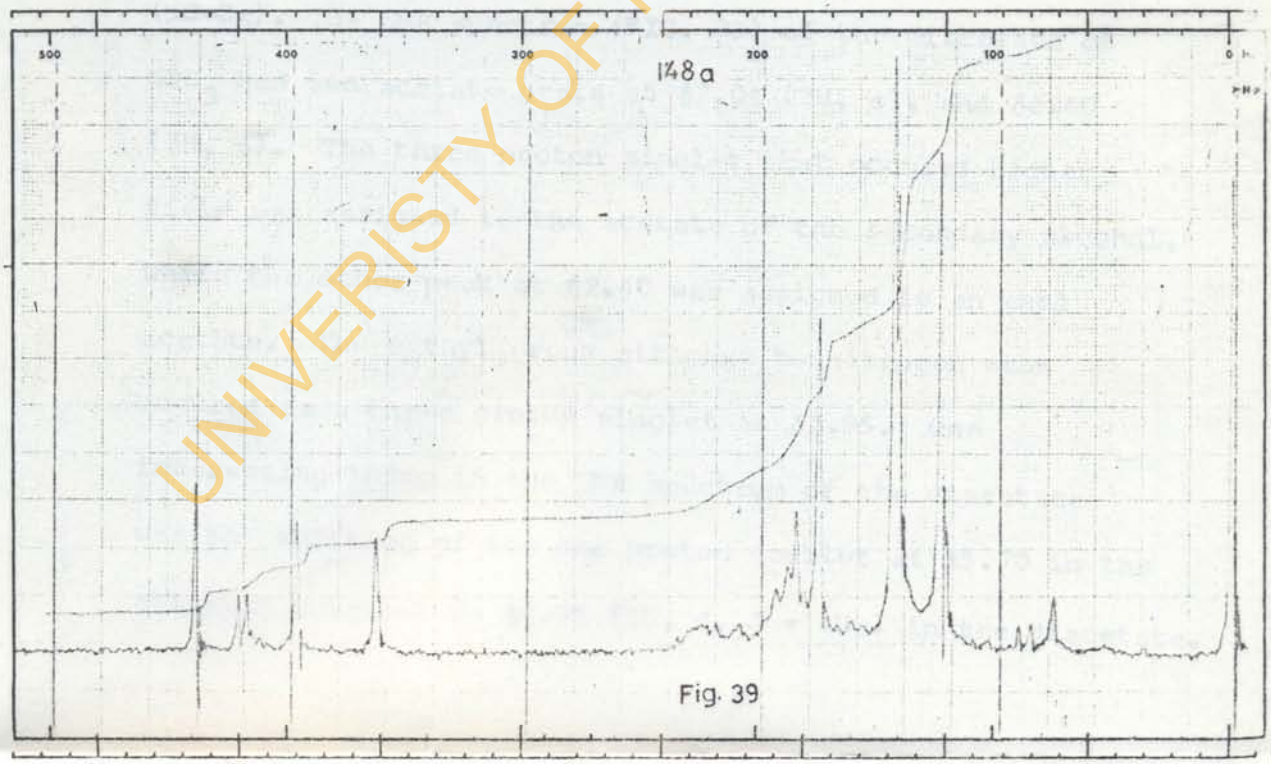


Fig. 39

MANUAL AUTO

SWEEP OFFSET (Hz): 5
SPECTRUM AMPLITUDE: 10
INTEGRAL AMPLITUDE: 50
SPINNING RATE (RPS): 40
SWEEP TIME (SEC): (250)
SWEEP WIDTH (Hz): (500)
FILTER: (2)
RF POWER LEVEL: 0.02 (.05)

SAMPLE: AcB_3
SOLVENT: $CHCl_3$
REMARKS:

DATE: 11/2/78 OPERATOR: J.O. Akinboye

60 MHz NMR SPECTRUM NO.

δ6.30 (1H, s) respectively. The proton signals at δ6.80 (1H, d) and δ12.40 (1H, s) were assigned to the secondary alcohol and the hydrogen-bonded phenolic hydroxyl groups respectively. The two signals disappeared with the addition of D_2O. Thus, the NMR spectrum suggested a total of 17 protons.

Acetylation of SRB_3 in a mixture of pyridine and acetic anhydride gave crystals of the diacetate (147; R^1 = R^2 = Ac), m.p. 223-225° (from MeOH/$CHCl_3$), which showed no hydroxyl absorption in the infrared spectrum. The IR spectrum (FIG. 38) of the diacetate further showed bands at ν_{max} 1750cm^{-1} ($-OCOCH_3$), 1660cm^{-1} (C=O), 1620cm^{-1} (>C=C<). The NMR spectrum (FIG. 39) of the diacetate of SRB_3 had two acetate peaks at δ2.01 (3H, s), and δ2.40 (3H, s). The three proton singlet that occured higher field was assigned to the acetate of the secondary alcohol, while the other peak at δ2.40 was assigned to an enol acetate. The methyl group attached to nitrogen atom occured as a three proton singlet at δ2.95. One interesting thing in the NMR spectrum of the diacetate was the shifting of the one proton doublet at δ5.70 in the starting material to δ6.95 (1H, d, J = 4Hz) in the diacetate.

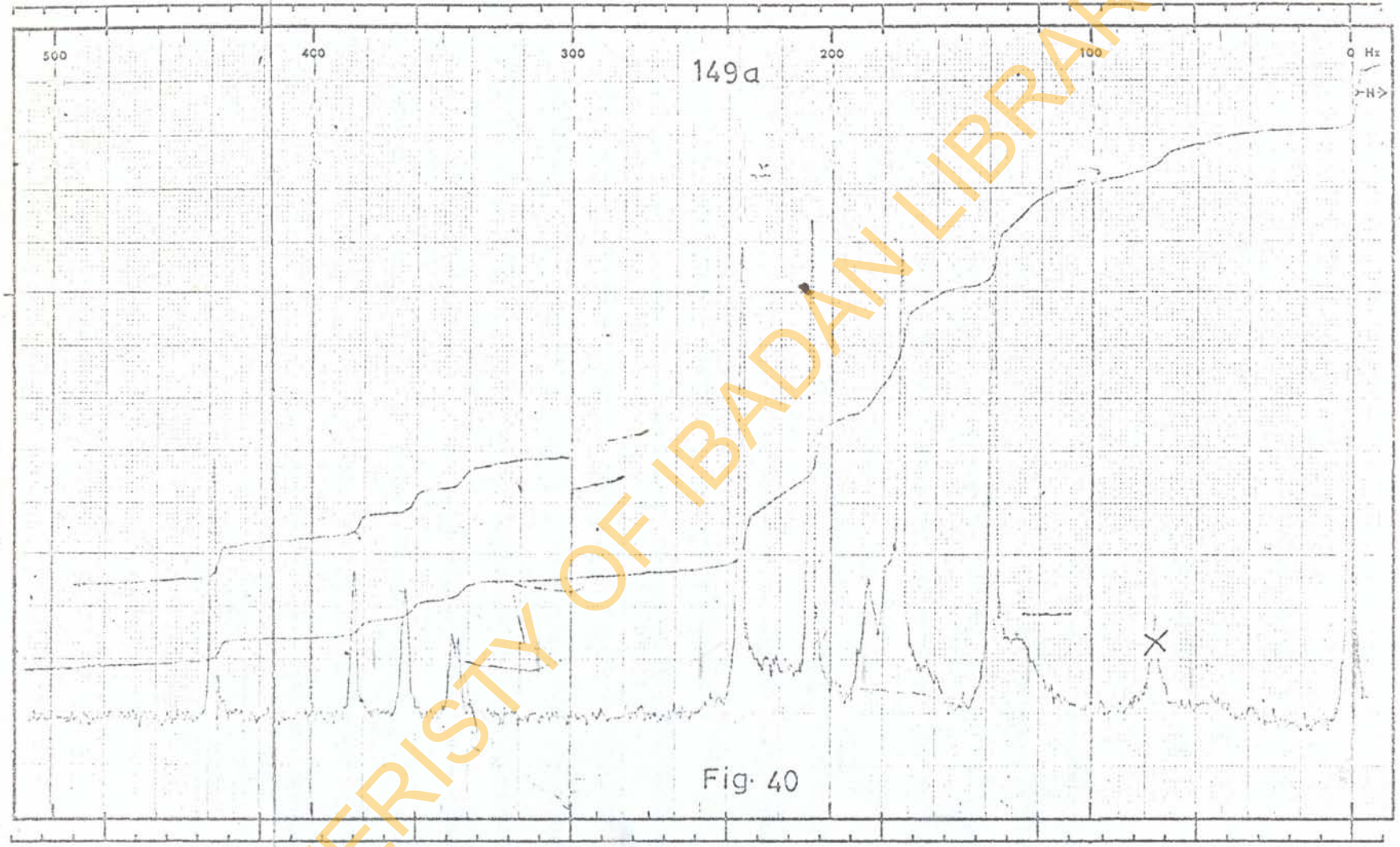


Fig. 40

SWEEP OFFSET (Hz): ---
SPECTRUM AMPLITUDE: 40
INTEGRAL AMPLITUDE: 3.0
SPINNING RATE (RPS): 40

MANUAL
SWEEP TIME (SEC): 50 100
SWEEP WIDTH (Hz): 25 50 100 250 500
FILTER: 1 2 3 4 5 6 7 8
RF POWER LEVEL: 0.05

AUTO ☐
(250)
(500)
(2)
(.05)

SAMPLE: MP3

REMARKS:
- X - Impurity

SOLVENT: $CDCl_3$

DATE: 11/2/78

OPERATOR: S.O. Adeboye

60 MHz NMR
SPECTRUM NO.

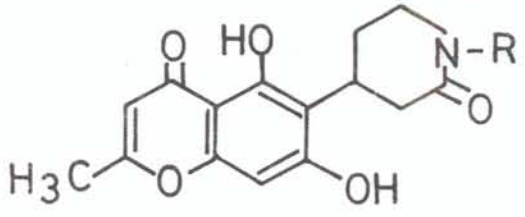
Diazomethane methylation of SRB_3 gave a mixture of two compounds, one corresponding to the monomethylether and the other to the starting material. The monomethylether was never obtained pure because no good separation could be effected on a column of silica gel since the R_f values of the starting material and the monomethylether were very close. However, this partial methylation product with diazomethane showed in the NMR spectrum the phenolic hydroxyl group at δ12.40 to be hydrogen-bonded while the secondary alcohol was methylated. The methoxyl proton signal appeared at δ3.48 as a three proton singlet.

Treatment of SRB_3 with a mixture of methyl iodide and silver oxide in chloroform[80] gave the monomethylether in poor yields. In an attempt to obtain the dimethylether of SRB_3, the above method was modified. Instead of stirring the reaction mixture for 10 hours, the reaction mixture was refluxed for 5 hours. This afforded the dimethylether of SRB_3, (147; $R^1=R^2=CH_3$) with m.p. 169-172°. The NMR spectrum (FIG.40) showed the following proton signals: δ2.35 (3H, s, $-CH_3$ at 2-position of γ-pyrone), δ2.93 (3H, s, assigned to $>N-CH_3$), δ3.48 (3H, s, non-aromatic $-OCH_3$), δ3.0 - δ3.8 (6H, m., CH_2 and CH), δ5.74 (1H, d, J = 4Hz, proton at the base of $-OCH_3$), δ6.05 (1H, s, proton

at 3-position of γ-pyrone) and δ6.40 (1H, s, aromatic proton). The NMR spectrum therefore suggested a total of 21 protons, which was in agreement with the expected molecular formula of the dimethylether of SRB_3, that is, $C_{19}H_{21}NO_6$.

As already mentioned under the discussion on SRB_4, the alkaline hydrolysis of SRB_3 gave as one of the products, a compound which was identical with 5,7-dihydroxy-2-methylchromone. The nitrogen-containing portion could not be isolated. The reason for this had earlier been given under the discussion on schumannificine (SRB_4).

It is worthwhile at this stage to compare both the physical constants and the spectral data of the two alkaloids, schumannificine (SRB_4) and N-methylschumannificine (SRB_3) with those of the two similar alkaloids, 46 and 47 isolated from Schumanniophyton problematicum[6] which were called piperidine-2-one alkaloids (no specific names were given to them).



46; R = H

47; R = CH_3

TABLE 3

	SCHUMANNIFICINE (SRB_4)	COMPOUND 46	N-METHYLSCHUMANNIFICINE (SRB_3)	COMPOUND 47
M.pt. (Recryst from)	234° (MeOH)	300-3°(EtOH)	208-209°(MeOH)	309-313°(EtOH/ Benzene
Molecular ion/Mol. Formula	M^+ 317; $C_{16}H_{15}NO_6$	M^+ 289; $C_{15}H_{15}NO_5$	M^+ 331; $C_{17}H_{17}NO_6$	M^+ 303; $C_{16}H_{17}NO_5$
UV(MeOH)	λnm log ε 220 4.13 225 4.13 253 3.86 260 3.88 280 3.88 290 3.85 310 3.95 320 3.96 333 3.97	λnm log ε 205 4.35 225 4.18 251 4.25 257 4.27 295 3.78 318 3.68	λnm log ε 220 4.12 225 4.12 253 3.89 260 3.90 277 3.89 290 3.90 310 3.95 320 3.96 335 3.98	λnm log ε 205 4.35 225 4.16 251 4.22 257 4.23 295 3.77 318 3.66
IR(cm^{-1})	ν_{max} (Nujol) 3150 (-NH) 1710 (w), 1650, 1620 (>C=C<) 1165, 1090(-O-)	ν_{max} (KBr) 3370 (-NH) 3300~2400(-OH) 1670, 1625 >C=C< 1600 (>C=C<)	ν_{max} (Nujol) 3360~3180 C-OH), 1670, 1630(>C=C<) 1575, 865 (aromatics)	ν_{max} (KBr) 3400~2400 (-OH), 1670, 1620(>C=C<) 1600 (>C=C<)

Table 3 contd.

	SCHUMANNIFICINE (SRB_4)	COMPOUND 46	N-METHYLSCHUMANNIFICINE (SRB_3)	COMPOUND 47
NMR(δppm)	d_5-pyridine 2.30 (allyl CH_3) 6.20 (vinyl H) 6.62(2H, s, aromatic and a proton at the base of -OH which was δ5.7 in $CDCl_3$)	d_6-DMSO 1.6-3.8 (7H) 2.38(allyl CH_3) 6.16(vinyl H) 6.30(arom.H) 7.53 (-NH) 10.88(7-OH) 12.9 (5-OH)	$CDCl_3$ 2.39 (allyl CH_3) 2.95 (N-CH_3) 3.2-3.6 (6H) 5.7(H at the base of OH) 6.06(vinyl H) 6.30(arom. H) 6.80 (-OH) 12.40 (-OH)	d_6-DMSO 1.6-3.8 (7H) 2.36 (allylCH_3) 2.84 (>N-CH_3) 6.14 (vinyl H) 6.26 (arom. H) 10.89 (7-OH) 12.90 (5-OH)
Mass Spectra	M/e 317 (M^+), 299,245,231 218,217,205 193,192 (base peak) 189,164,163, 149, 124.	m/e 289 (M^+) 272,245,244 231,229,219, 218,217,216 205(base peak), 193,192,189.	m/e 331 (M^+) 313,303,245, 219,218,208 205 (base peak) 193,192,189, 164,112,69.	m/e 303 (M^+), 272,245,244 231,218,217 216,205 (base peak), 193, 192.

Comparison of the physical and spectral data represented in Table 2 for the reported piperidine-2-one alkaloids, 46 and 47 and the new alkaloids schumannificine (SRB_4) and N-methylschumannificine (SRB_3) confirmed that the two sets of alkaloids were not identical. However, the similarities observed in their fragmentation patterns is a pointer to the relationship between them. The chromone portion of the two sets of alkaloids is clearly established in the NMR spectra of the alkaloids by the absorptions at δ2.38 (3H, s, CH_3 at 2-position of γ-pyrone), δ6.16 (1H, s, proton at 3-position of γ-pyrone) and δ6.30 (1H, s, aromatic proton) for 46 and at δ2.30, δ6.20 and δ6.62 (shifted downfield by deuteropyridine) for schumannificine (SRB_4); at δ2.36, δ6.14 and δ6.26 for 47 and at δ2.39, δ6.06 and δ6.30 respectively for N-methylschumannificine (SRB_3). Also, in the mass spectra, apart from schumannificine, others, 46, 47 and N-methylschumannificine have m/e 205 as the base peak while the following fragment ions are common to the four compounds, m/e 245, 218, 193 and 192. The fragmentation patterns of the compound are similar in some respects as shown by the fragment ions quoted above.

154a

DEHYDROSCHUMANNIFICINE

(SBR_2)

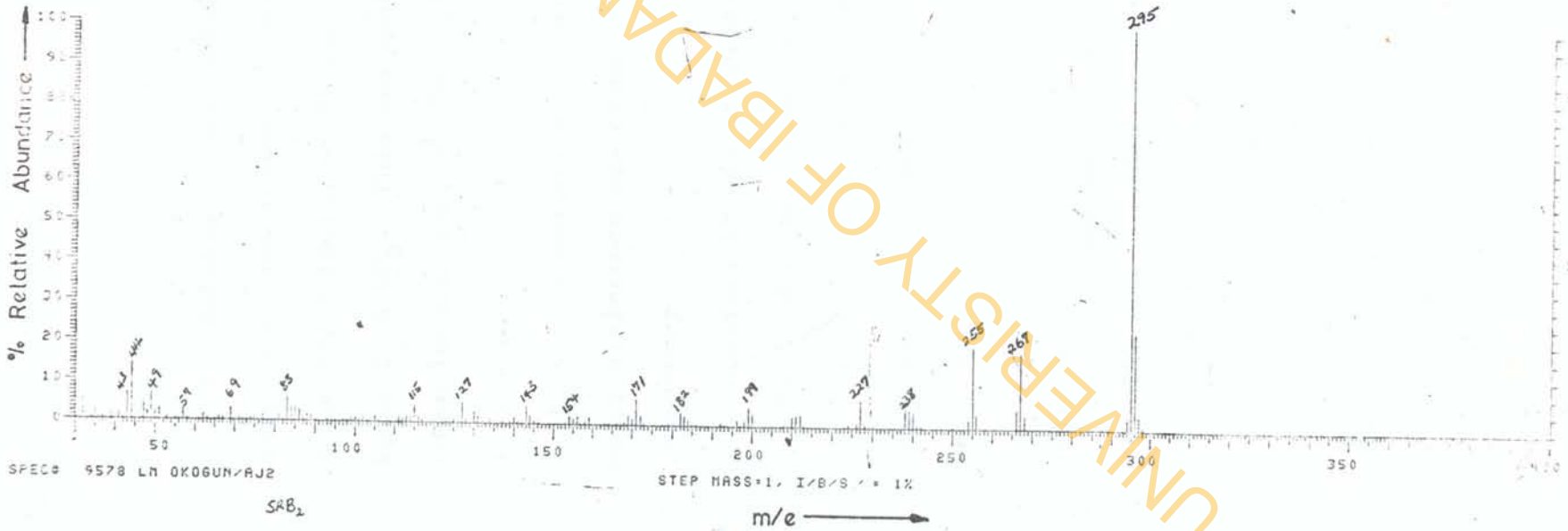


Fig 41

The remaining three alkaloids, SRB_2, SRB_3' and SRB_3'' had a few things in common. They had the same molecular formula, which was obtained from the microanalysis to be $C_{16}H_9NO_5$. This was confirmed to be consistent with the molecular ion, M^+ 295, given by the mass spectrum. It was quite clear from the comparison of their mass spectra that the fragmentation patterns were very similar. The major difference was found in the percentage relative abundance.

However, it was abundantly clear from their m.p., IR, and UV spectra that these alkaloids were different, although they might be related. The NMR spectra of the three alkaloids could not be obtained because of solubility problem.

III. DEHYDROSCHUMANNIFICINE (SRB_2).

SRB_2 had a m.p. 290-292°. It was recovered from the treatment of a mixture of SRB_2 and SRB_3'' with aqueous ammonia and was shown to contain nitrogen (microanalysis). It gave positive alkaloid test with Dragendorff's reagent and positive phenol test with ferric chloride solution.

The mass spectrum (FIG.41) indicated a parent peak at M^+ 295 (100 - base peak), which was in accordance with the molecular formular. The following prominent peaks were observed

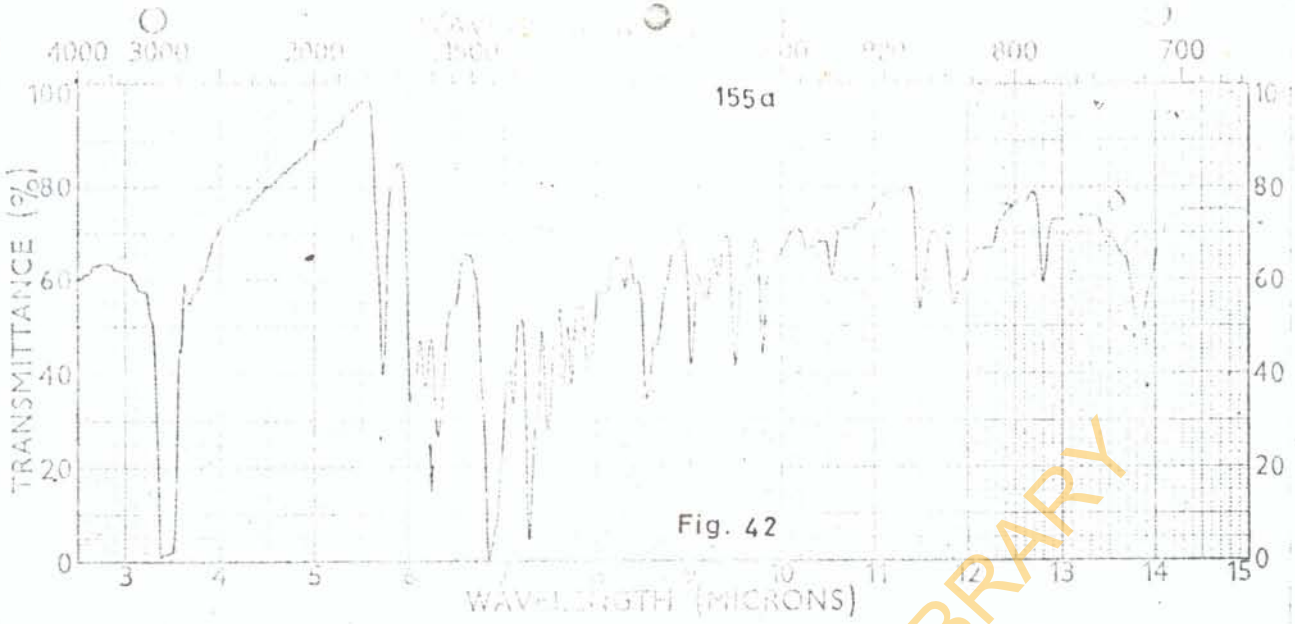


Fig. 42

SAMPLE SRB2	PHASE Nujol	SCAN SPEED FAST SLIT NORMAL
	SOLVENT	OPERATOR ADEBOYE DATE 23-4-79
	CONC.	REMARKS
	CELL PATH	
ORIGIN	REFERENCE	

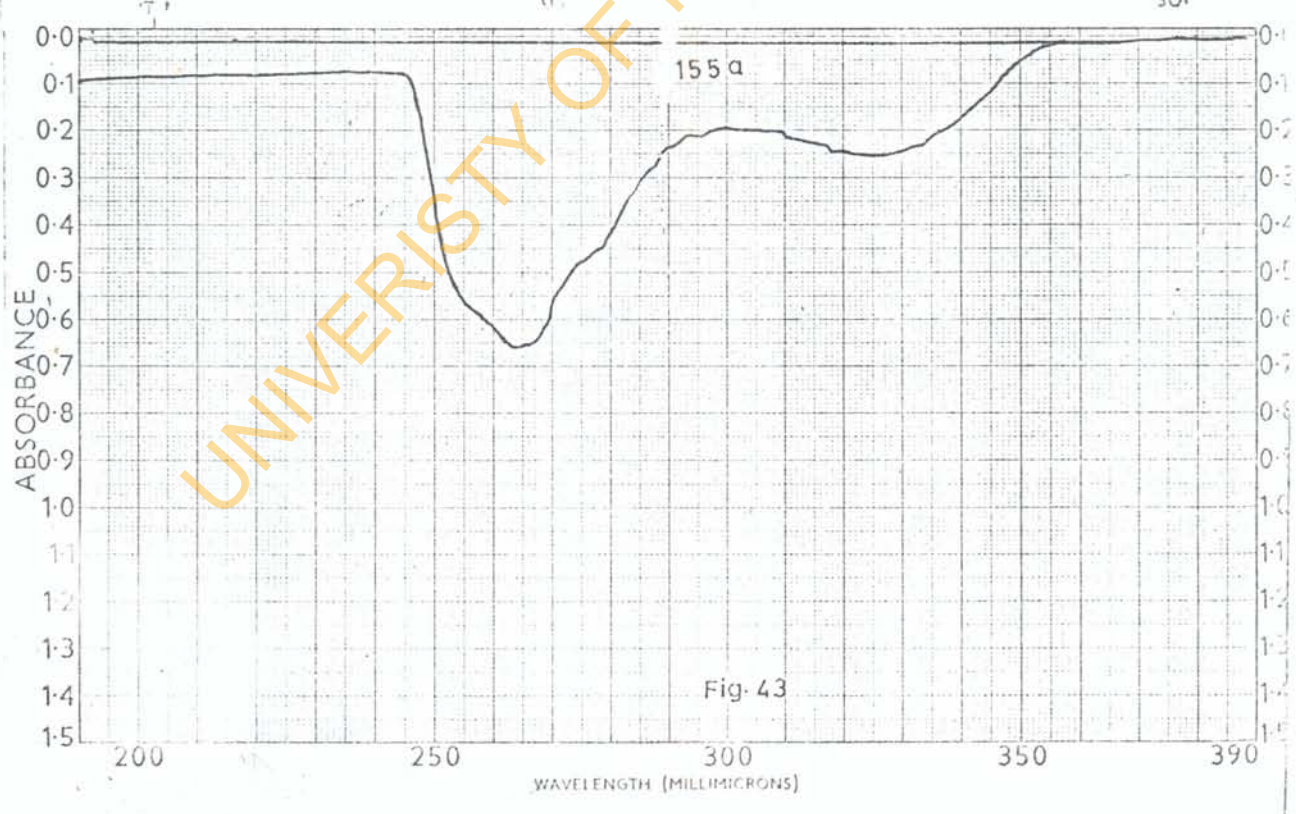


Fig. 43

SAMPLE SRB2	CURVE NO.	SCAN SPEED FAST	OPERATOR ADEBOYE
	CONC. 6 mg/1000 cm³	SLIT	DATE 23-7-81
ORIGIN	CELL PATH	REMARKS	
SOLVENT CHCl₃	REFERENCE		

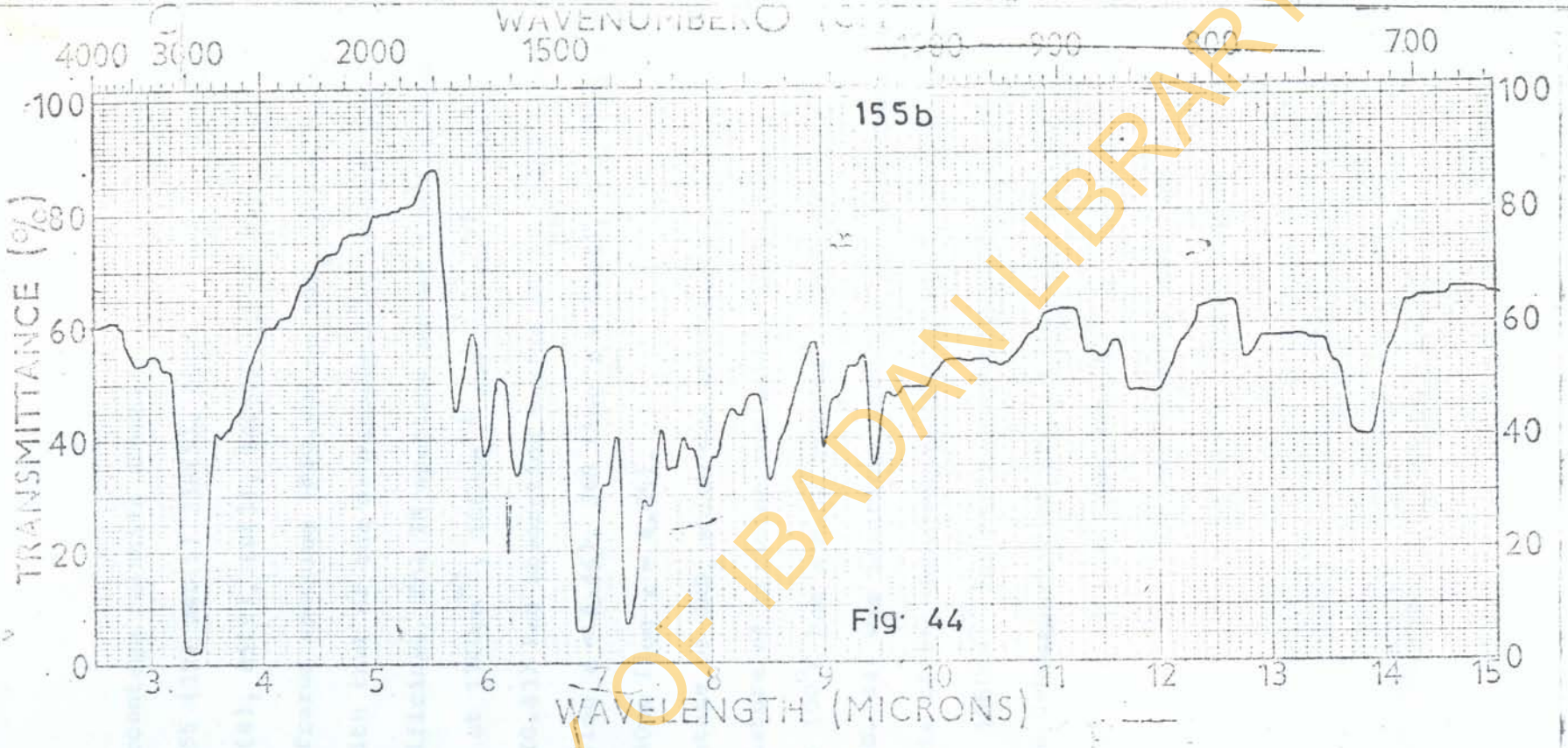


Fig. 44

SAMPLE AC B_2 By treatment with Ac_2O/Pyridine at 100°C.

PHASE N

SOLVENT

CONC.

CELL PATH

REFERENCE

ORIGIN

SCAN SPEED FAST SLIT NORMAL

OPERATOR ADEBOYE DATE 23/10/79

REMARKS

NO.

PART NO. 137E - 1281

PERKIN - ELMER LIMITED, BEACONSFIELD, BUCKS

with the percentage relative abundances put in the parenthesis: 268 (18), 255 (19) 240(5) 238(5), 277(7), 199(5), 171(6), 127(5), 115(4), 85(5), 69(3), 44(13) and 43(5).

The infrared spectrum (FIG.42) of SRB_2 proved to be identical with that of the dehydrogenation product 142 of schumannificine. The IR spectrum showed the carbonyl absorptions at $1740cm^{-1}$, $1660cm^{-1}$ and $1620cm^{-1}$. The UV spectrum (FIG.43) had absorption maxima at λ_{max} 257 (log ϵ = 4.38), 263 (log ϵ = 4.44), 268 (log ϵ = 4.44), 321 (log ϵ = 4.05) and 330nm (log ϵ = 4.05).

The acetate of SRB_2 which was obtained by heating SRB_2 in a mixture of pyridine and acetic anhydride for six hours at 100°C had a m.p. 282-284°. The infrared spectrum (FIG. 44) was identical with the infrared spectrum of the acetate of the dehydrogenation product, showing bands at $1750cm^{-1}$, $1660cm^{-1}$ for the carbonyl absorptions. The m.p.s for the dehydrogenation product 142 and its acetate were respectively, 294-296° and 285-287°C.

From the above spectral and physical data and comparison with the dehydrogenation product and its acetate, SRB_2 was concluded to be identical with the product of dehydrogenation 142 and was therefore named dehydroschumannificine.

156a

SRB_3^1

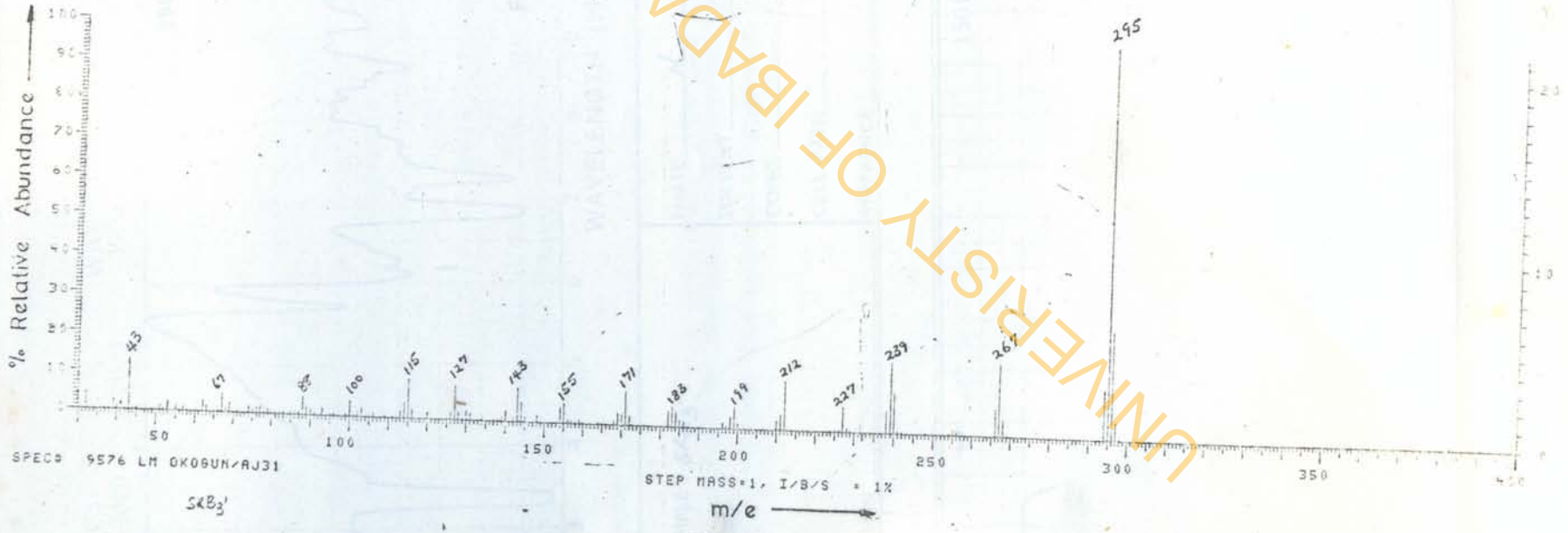


Fig. 45

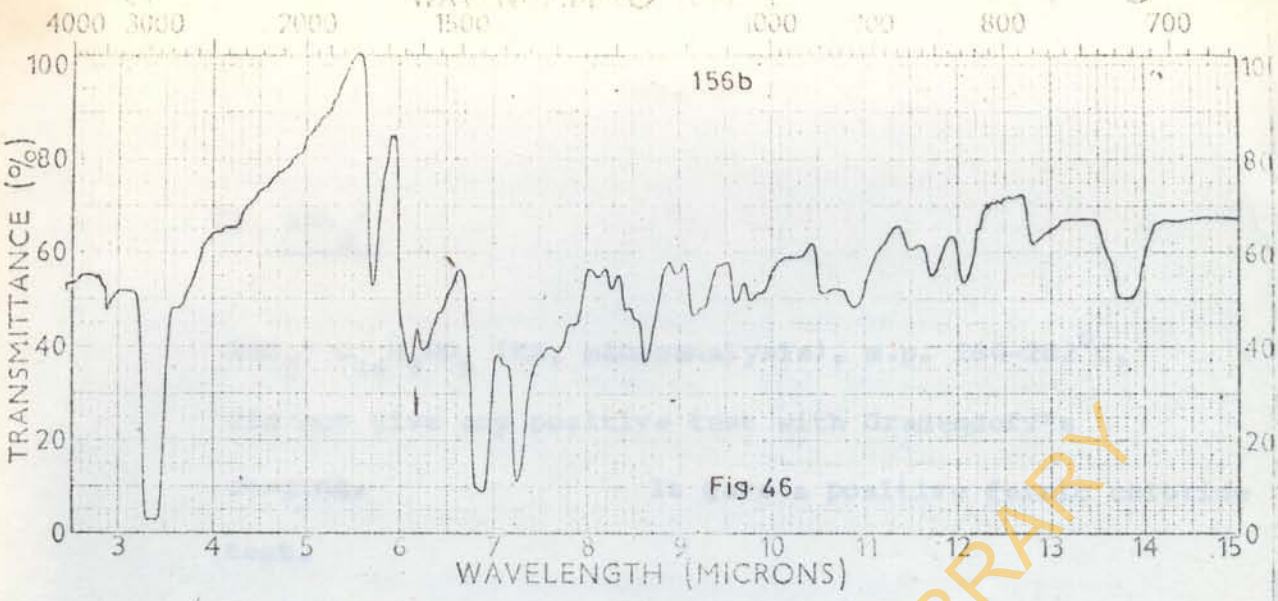


Fig. 46

SAMPLE SRB3'	PHASE N	SCAN SPEED FAST SLIT NORMAL
	SOLVENT	OPERATOR ADEBOYE DATE 24/10/79
	CONC.	REMARKS
	CELL PATH	
ORIGIN	REFERENCE	

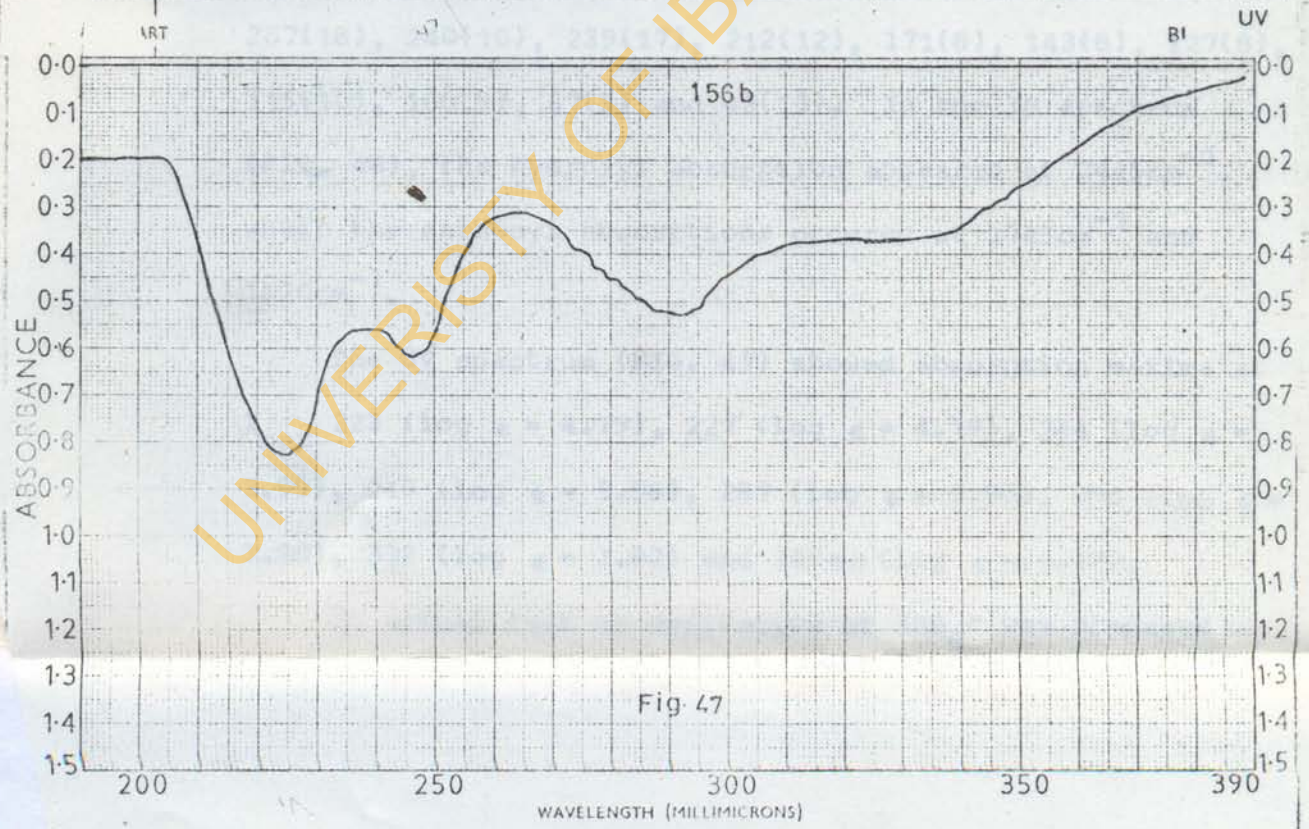


Fig. 47

SAMPLE SRB3'	CURVE NO.	SCAN SPEED FAST	OPERATOR ADEBAYO
	CONC. 12 mg/1000 cm³	SLIT	DATE 15-7-81
ORIGIN	CELL PATH	REMARKS	
SOLVENT	REFERENCE		

IV SRB_3'

SRB_3' $C_{16}H_9NO_5$ (MS, microanalysis), m.p. 280-282°C, did not give any positive test with Gradendoff's reagent. It gave a positive ferric chloride test.

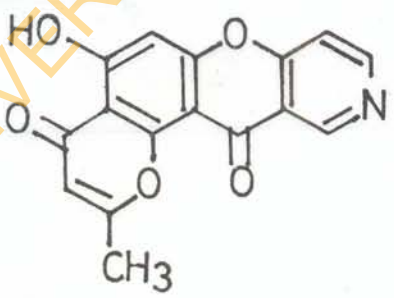
From the mass spectrum (FIG.45), the molecular ion, M^+, was obtained to be 295 (100% base peak) which agreed with the molecular formula. Apart from the parent ion, other prominent peaks are shown below with the percentage relative abundances shown in the parentheses. 294(12.4), 267(18), 240(10), 239(17), 212(12), 171(8), 143(8), 127(8), 115(10), 100(5), 67(5) and 43(13). In the IR spectrum (FIG. 46), the hydroxyl absorption appeared at 3445cm^{-1}, while the carbonyl absorptions occured at 1745cm^{-1} and 1620cm^{-1}.

The UV spectrum (FIG. 47) showed absorption maxima at λ_{max} 223 (log ϵ = 4.19), 227 (log ϵ = 4.19), 244 (log ϵ = 3.99), 249 (log ϵ = 3.98), 287 (log ϵ = 3.90), 294 (log ϵ = 3.90), 332 (log ϵ = 3.82) and 340 nm (log ϵ = 3.77).

In actual fact no derivative of SRB_3' was prepared

and no degradative work was carried out because SRB_3' occured in trace amount.

The fragmentation pattern of SRB_3' was similar to that of dehydroschumannificine 142 but there was a slight difference in their IR spectra. SRB_2 showed carbonyl absorptions at $1740cm^{-1}$, $1660cm^{-1}$ and $1620cm^{-1}$ while SRB_3' showed its carbonyl absorptions at $1745cm^{-1}$ and $1620cm^{-1}$. It could be suggested then that the γ-pyrone carbonyl absorption was reduced to 1620 due to hydrogen-bonding. On the basis of the similarity in the fragmentation patterns of SRB_2 and SRB_3' and the low carbonyl absorption of the γ-pyrone carbonyl absorption, structure 148 was proposed for SRB_3'.



148

158a

SRB_3^{II}

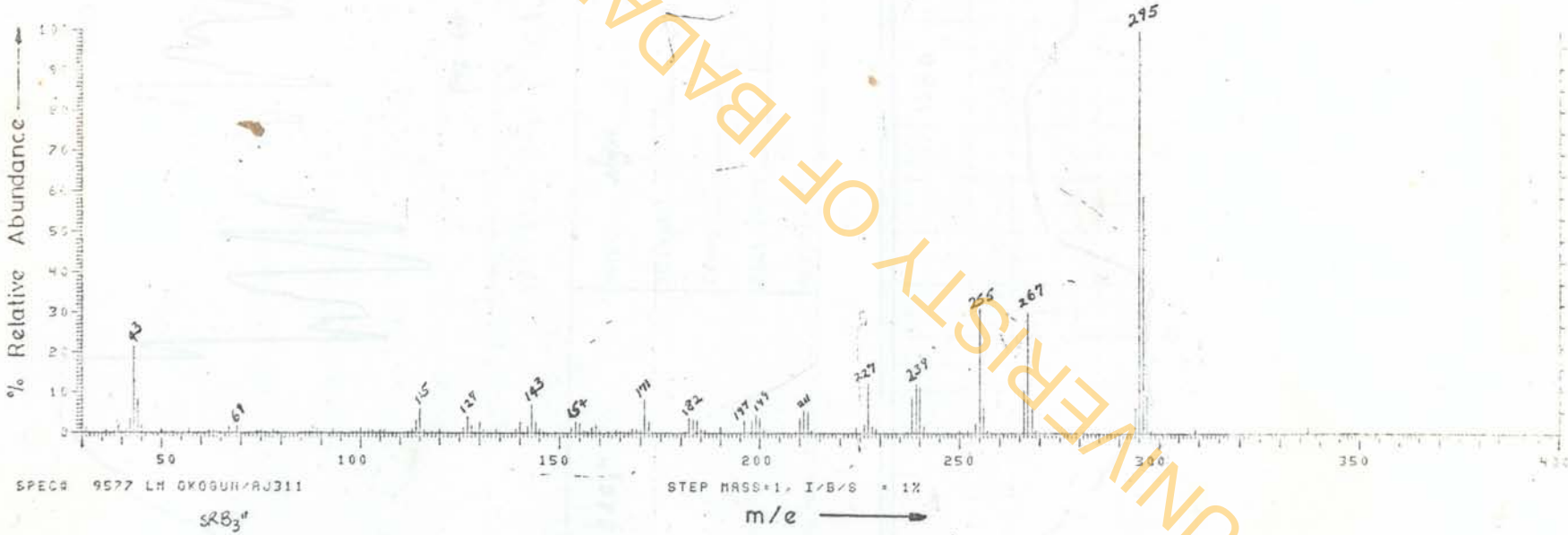


Fig. 48

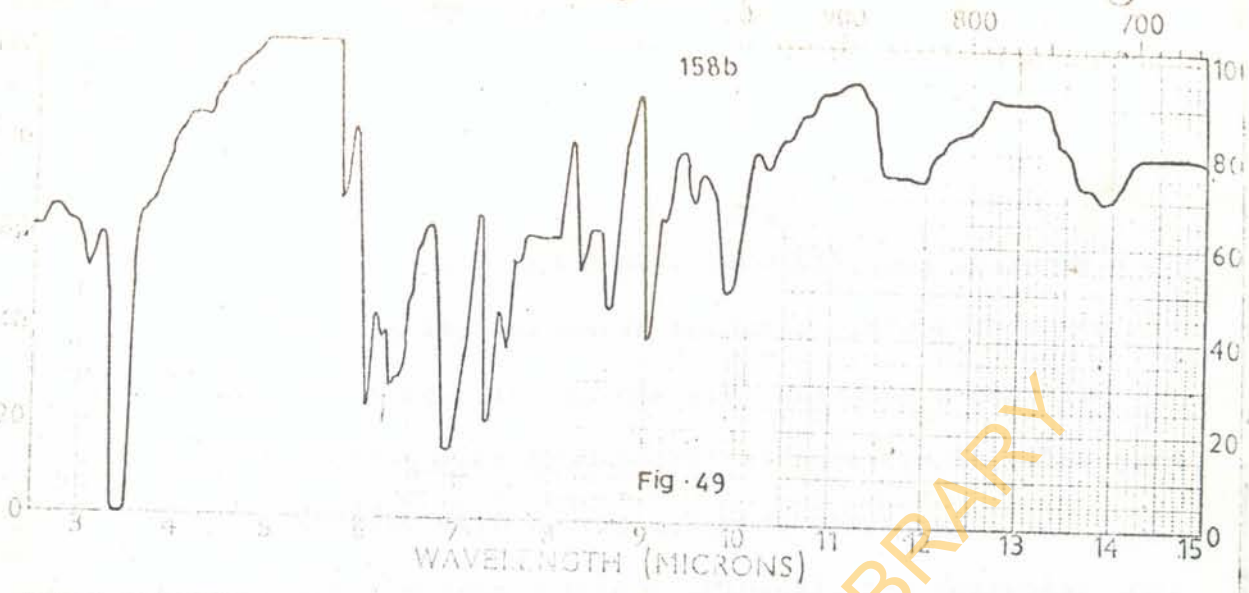
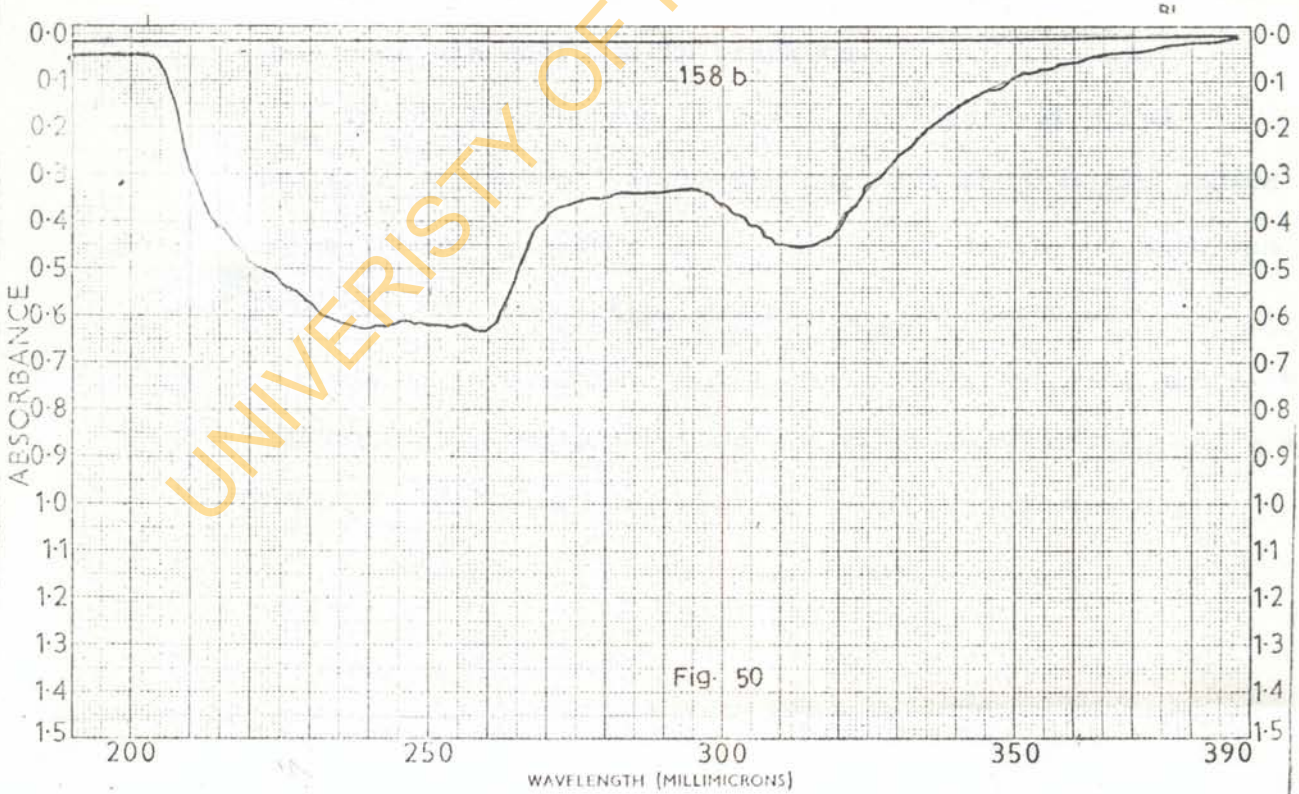


Fig · 49

SAMPLE SRB3"	PHASE Nujol	SCAN SPEED Fast SLIT Normal
	SOLVENT	OPERATOR J.O. Adeoye DATE 14/1/78
	CONC.	REMARKS
ORIGIN	CELL PATH	
	REFERENCE	



Fig· 50

SAMPLE SRB3"	CURVE NO.	SCAN SPEED FAST	OPERATOR ADEBOYE
	CONC. 12 mg /100cm³	SLIT	DATE
ORIGIN	CELL PATH	REMARKS	
SOLVENT MeOH	REFERENCE		

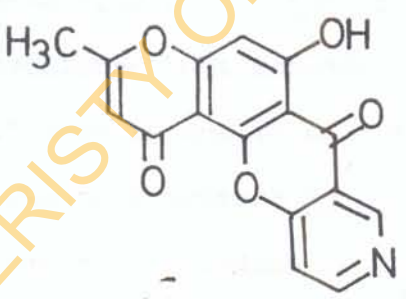
V. SRB_3"

SRB_3" which had a m.p. 248-251°, was assigned $C_{16}H_9NO_5$, as its molecular formula. It was shown to contain nitrogen (microanalysis) and gave a positive alkaloid test with Dragendorff's reagent. It also gave positive test with ferric chloride solution.

From the mass spectrum (FIG.48), the molecular ion, M^+, was 295 (100%-base peak), which was in accordance with the molecular formula. It showed in addition to the parent ion, the following peaks, 267(69), 266(11), 260(31), 240(11), 239(11.5), 238 (8.6), 227 (11.8), 199(5), 171(8), 143(7), 115(6), and 39(8). The percentages of the relative abundance are enclosed in brackets.

In the IR spectrum (FIG.49), the hydroxyl group appeared at 3200cm^{-1} while the carbonyl absorptions showed up at 1680cm^{-1}, 1660cm^{-1}. . In the UV spectrum (FIG.50), the absorption maxima, λ_{max} appeared at 225(sh, log ϵ =3.96), 232 (log ϵ = 4.14), 240 (log ϵ = 4.16), 280 (log ϵ = 4.03), 315 (log ϵ = 4.03) and 323m(sh, log ϵ = 3.90). The values were similar to those obtained for 5,7-dihydroxy-2-methylchromone, schumannificine (SRB_4) and N-methylschumannificine (SRB_3).

The fragmentation pattern of SRB_3" was similar to those of SRB_2 and SRB_3' but there was a marked difference in their IR spectra. SRB_2 showed its carbonyl absorptions at 1740cm^{-1}, 1660cm^{-1}, the carbonyl absorptions for SRB_3' appeared at 1745cm^{-1} and 1640cm^{-1} but those of SRB_3" appeared at 1680cm^{-1}, and 1660cm^{-1}. In SRB_3", the characteristic chromone carbonyl absorptions were shown at 1660cm^{-1}, but 1680cm^{-1} which was low compared with 1740cm^{-1} for SRB_2 could have been as a result of hydrogen-bonding effect on the carbonyl in the γ-position to the nitrogen atom, hence structure 149 was proposed for SRB_3".



149

The synthesis of dehydroschumannificine 142, and further chemical investigations are expected to continue on SRB_3' and SRB_3" to allow the assignments of their correct structures, if at all they differ from the proposed structures.

CONCLUSION

Chromones and the related alkaloids.

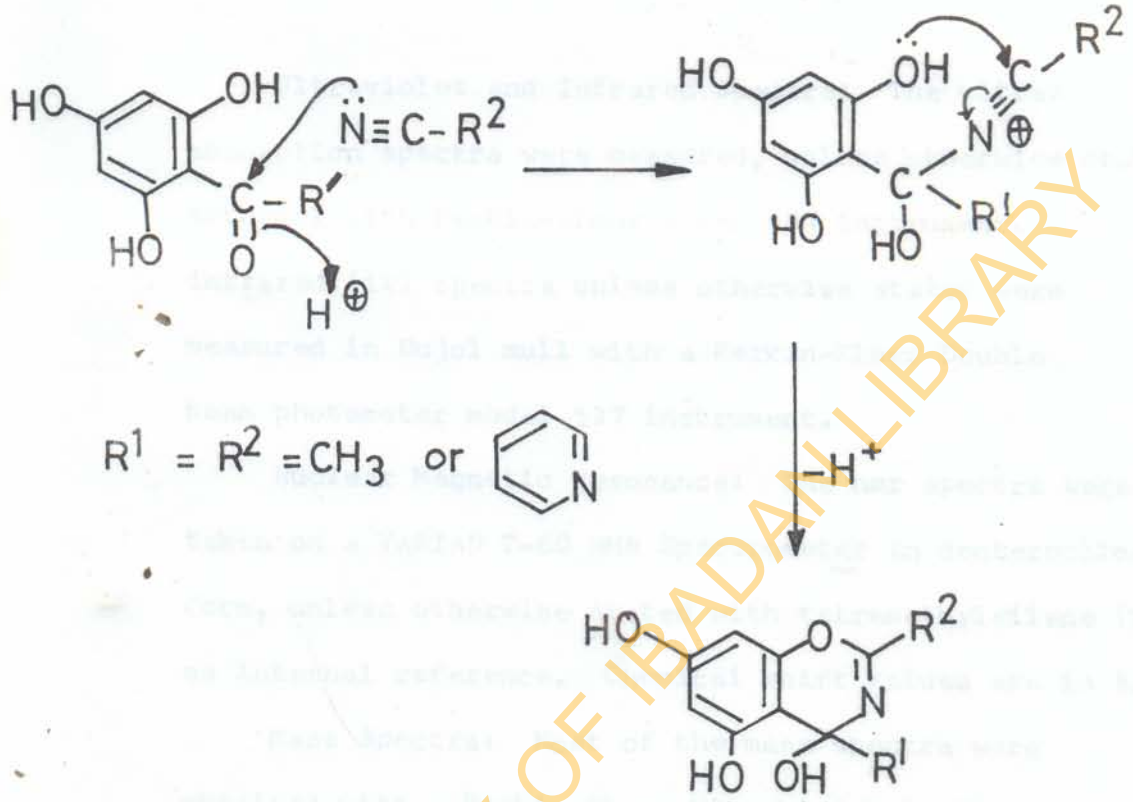
Chromones rarely occur in Rubiaceous plants. The most outstanding of the chromones which has been reported to occur in Rubiceae is 5,7-dihydroxy-2-methylchromone 97. In Schumanniophyton problematicum[6] and S. magnificum 5, 7-dihydroxy-2-methylchromone occured with other related alkaloids. The co-existence of these compounds is of biogenetic significance. The biosynthesis of 5,7-dihydroxy-2-methylchromone and other chromones has been well documented[54-56]. Although, no nicotinic acid derivatives had been reported isolated from S. problematicum and s. magnificum, it could be speculated that dehydroschumannificine 142, SRB_3' and SRB_3'' were formed in the plants by acylation of 5,7-dihydroxy-2-methylchromone 97 with nicotinic acid derivatives. Subsequent reduction and hydroxylation of the pyridine ring of 142 would give schumannificine (146; $R^1=R^2=R^3=H$) and N-methylation of schumannificine would afford N-methylschumannificine (147; $R^1=R^2=H$).

Hoesch Synthesis.

This is an established method of synthesizing 2,4,6-trihydroxyacetophenone[96]. However, the method has been

very useful in introducing the keto group into the phloroglucinol residue via the nitrile. Various nitriles have been employed in this type of synthesis, ranging from aliphatic to aromatic ones, but there was no report of the use of nicotinonitrile. When the reaction between phloroglucinol and nicotinonitrile was carried out in ether, there was no reaction. This might be due to solubility problem, but 1,2-dimethoxyethane served as a better solvent under this condition.

When 2,4,6-trihydroxyacetophenone and 2,4,6-trihydroxynicotinophenone were treated with nicotinonitrile and acetonitrile respectively, the expected product could not be obtained. This could be due to two factors. The first being the deactivating effect of the keto group on the benzene ring. The second factor is attributed to the possibility of attack on the carbonyl carbon in such an acidic medium. The process of such an attack is shown in scheme 21.



Scheme 21

If the process in scheme 21 was predominating over the attack of $RC^+ = NH$ on benzene ring, then the expected product could not be obtained.

EXPERIMENTAL

Ultraviolet and Infrared Spectra: The ultraviolet (UV) absorption spectra were measured, unless otherwise stated in methanol with Perkin-Elmer model 137 instrument. The infrared (ir) spectra unless otherwise stated were measured in Nujol mull with a Perkin-Elmer Double beam photometer model 137 instrument.

Nuclear Magnetic Resonance: The nmr spectra were taken on a VARIAN T-60 NMR Spectrometer in deuterochloroform, unless otherwise stated with tetramethylsilane (TMS) as internal reference. Chemical shift values are in δ.

Mass Spectra: Most of the mass spectra were obtained with a Perkin-Elmer Hitachi R.M.U. 6E instrument and at the Institute of Organic Chemistry, at the Technical University, Darmstadt, W. Germany. Microanalyses were carried out by Mr. P.I. Mowete at the Chemistry Department, University of Ibadan and at the Institute of Organic Chemistry, Technical University, Darmstadt, W. Germany. All melting points (m.ps.) were taken on a Reichert Akt Microscrope Hot plate model and were uncorrected.

Thin plate Chromatograms for preparative thin layer chromatography (T.l.c.) were made by mixing 80gm of Merck Kieselgel 60F 254+366 with 180cm^3 of distilled water and spreading the aqueous slurry on a 20 x 100cm plate which was dried at 120^{o}C for 2 hours. Analytical thin layer chromatography was performed on a slurry of kieselgur G. Merck silica gel (70-230 mesh) and the silica gel used for column chromatography refers to merck 0.05mm-0.2mm mesh. Solvents were purified using standard procedures and petroleum ether, unless otherwise stated, refers to petroleum spirit (60-80^{o}C). Dragendorff's reagent refers to a solution of Bismuth sub-nitrate (1.7g) in a 100cm^3 of H_2O-HOAc (4:1) and potassium iodide (40g) in 100cm^3 of water.

Extraction of the root-bark.

The root-bark obtained from the tap-root of Schumanniophyton magnificum (Harms) were ground or pulverised. The ground material (4kg) was then successfully extracted in a Soxhlet extractor with hexane (24hrs) and methanol (24hrs).

Treatment of the methanol extract.

The methanol extract which was concentrated at reduced pressure was left at room temperature. After two days, a precipitate settled with an oily upper layer. The oily layer (500cm^3) was decanted and transferred to a separating funnel. This was extracted with hexane, ether and chloroform in that order.

Further extractions were limited to the use of hexane and chloroform since all the compounds in ether extract were found in chloroform extract, except one, which formed the major component of hexane extract (t.l.c.). The hexane extracts were concentrated and preserved. The oily layer was extracted several times with chloroform and the various extracts evaporated on a water-bath. These various extracts yielded brown crude solids, which were left for some days to allow complete removal of the solvent. About 20.8g of the crude solid was recovered from 4kg of material which included the crude solid recovered from the precipitate when extracted several times with chloroform. The water soluble oil (80g) left after extracting the oily layer with chloroform was preserved.

Alkaloid test.

Pilot thin layer chromatography plate of the chloroform extract was developed in a mixture of $CHCl_3$/EtOAc (3:1). The dried plate was sprayed with Dragendorff's reagent. The test proved positive for four compounds, SRB_2, SRB_3, SRB_3', SRB_3'' and SRB_4. The positive colour was red. SRB_3' which did not give the positive colour on spraying with Dragendorff's reagent proved to be a nitrogen-containing compound (from microanalysis).

Analytical Thin layer Chromatography.

The analytical thin layer chromatography plates used in piloting the reactions were prepared by spreading a slurry of silica gel (70-230mesh) as evenly as possible onto the microplates (slides). The slurry was prepared by mixing silica gel with water (ratio 1:2). These were dried or cultivated in the oven set at 110°.

Spots of the solution of the required sample were made on the t.l.c. plates by means of micro capillary tube (spotting tube). The plates were developed in a mixture of solvents e.g. chloroform and ethylacetate (3:1).

After the development, plates were removed from the solvent mixture and left to dry. These plates were finally transferred into a beaker containing iodine crystals for visualization of the compounds.

Treatment of crude solid with dilute HCl.

Crude solid (500mg) was dissolved in chloroform (200cm^3). Dilute hydrochloric acid (100cm^3) was added and well shaken. The chloroform layer (from t.l.c.) contained all the compounds almost in the same proportions.

The acid layer was basified with dilute ammonia solution. There was no immediate precipitate. It was shaken and kept in the cold. The compounds in the basic solution were extracted into chloroform. All the compounds were represented in the chloroform extract. The solid recovered on evaporation of the extract looked exactly like the crude solid but much more purer.

Column Chromatography of the crude solid.

Crude solid (16g) was dissolved in chloroform (100cm^3). To this solution of the crude solid in chloroform was added silica gel and made into a slurry. It was then introduced into a column of silica gel.

Elution with Ether/Hexane (1:1) afforded SRB_1 (the chromone) as white crystals (1.5g) m.p. 274-276°. {Found:C:62.78;H: 4.54. $C_{15}H_{10}O_4$ requires for C; 62.50; H: 4.20}. Molecular ion, M^+ at m/e 254 with other prominent peaks at 164, 163, 152, 136, 124, 96 and 69. ν_{max} (KBr) 3420-2620cm^{-1} (-OH groups), 1660, (-C=O), 1560, 1500, 890, 845 and 820cm^{-1} (substituted aromatic rings). 1165cm^{-1} (ether linkage). λ_{max} (MeOH), 217nm (log ε = 4.21), 227 (log ε = 4.18), 251 (log ε = 4.21), 257 (log ε = 4.22), 295 (log ε = 3.83) and 325 (log ε = 3.68).

δppm (d_6-DMSO), 2.27 (3H, s, $-CH_3$), 6.08 (1H, s, proton at 3-position of γ-pyrone), 6.18 (1H, d, J = 2Hz, aromatic), 6.28 (1H, d; J = 2Hz, aromatic), 12.7 (1H, s, disappeared with D_2O).

Further elution with $CHCl_3$/Ether (1:1) gave a mixture of SRB_3 and SRB_3' (800mg), which on fractional crystallization from methanol afforded SRB_3 (500mg), m.p. 208-209°. {Found, C: 59.39; H: 6.02; N: 4.15. $C_{17}H_{17}NO_6$.MeOH required C: 59.49; H: 5.83; N: 3.86}.

M^+ 331 (97%), m/e 313, 303, 219, 218, 208, 205, 193, 192 189, 164, 112 and 69.

ν_{max} 3360~3180cm^{-1} (-OH group), 1670, 1630cm^{-1} (-C=O), 1575, 865, 845 and 820cm^{-1} (aromatic rings).

λ_{max} (MeOH), 220nm (log ε = 4.12), 225 (log ε = 4.12), 253 (log ε = 3.89), 260 (log ε = 3.90), 277 (log ε = 3.89, sh), 290 (log ε = 3.90); 310 (log ε = 3.95, sh), 320 (log ε = 3.96) and 335mμ (log ε = 3.98, sh).

δppm ($CDCl_3$) 2.39 (3H, s, $-CH_3$), 2.95 (3H, s, $-N-CH_3$), 3.2 - 3.6 (6H, m, CH_2 and CH), 5.7 (1H, d, J = 4Hz, proton at the base of hydroxyl group), 6.06 (1H, s, proton at 3-position of γ-pyrone), 6.30 (1H, s, aromatic), 6.8 (1H, d, dissappeared with D_2O) and 12.4 (1H, s disappeared with D_2O).

Elution with chloroform/Ether (4:1), gave a little amount of pure SRB_2 (50mg) while continuous elution with the same solvent mixture, chloroform/Ether (9:1) and pure chloroform gave in both cases, a mixture of SRB_2 and SRB_3" (1.8g). Further elution with methanol/ chloroform (1:1) gave pure SRB_4 (300mg), m.p. 234°. {Found C: 60.84, H: 4.86, N: 4.47. $C_{16}H_{15}NO_6$ required C:60.56, H: 4.77, N: 4.41}. M^+ 317 (12.1%), m/e 299, 245, 231, 218, 217, 205, 192(100 - base peak), 189 164, 163, 149 and 124.

ν_{max} (Nujol) 3150cm^{-1} (-N-H), 1710 (W), 1650, (-C=O), 1165, 1090cm^{-1} (ether linkage), 1580, 845 and 720cm^{-1} (substituted aromatic rings).

λ_{max} (MeOH), 220 (log ϵ = 3.86), 260 (log ϵ = 3.88), 280 (log ϵ = 3.88, sh), 290 (log ϵ = 3.85), 310 (log ϵ = 3.95, sh), 320 (log ϵ = 3.96) and 333nm (log ϵ = 3.97, sh).

δppm (d_5-C_5H_5N), 2.30 (3H, s; $-CH_3$), 6.20 (1H, s, proton at 3-position of γ-pyrone), 6.62 (2H, s, aromatic proton and a proton at the base of hydroxyl group).

SRB_3' was obtained as pure needle-like crystals by recrystallizing from chloroform, the residue compound left after fractional crystallization of SRB_3 from a mixture of SRB_3 and SRB_3'. Pure SRB_3' (30mg) had a m.p. 280°-282°. {Found C: 65.25, H: 3.24; N: 4.46. $C_{16}H_9NO_5$ required C: 65.09, H: 3.07, N: 4.74}.

M^+ 295 (100-base peak), m/e 294, 267, 240, 239, 212, 171, 143, 127, 115, 100, 69, 43.

ν_{max} (Nujol) 3445cm^{-1} (-OH group), 1745, 1640cm^{-1} (C=O), 1600cm^{-1} (-C=C-, aromatic), 1160, 1080cm^{-1} (ether linkages), 915, 850 and 830cm^{-1} (substituted aromatic rings).

λ_{max} (MeOH) 223 (log ϵ = 4.19), 227 (log ϵ = 4.19), 244 (log ϵ = 3.99), 249 (log ϵ = 3.98), 287 (log ϵ = 3.90), 294 (log ϵ = 3.90), 332 (log ϵ = 3.82) and 340nm (log ϵ = 3.77).

Treatment of a mixture of SRB_2 and SRB_3'' with aqueous ammonia.

Aqueous ammonia ($10cm^3$) was added to a mixture of SRB_2 and SRB_3'' (200mg). The mixture was allowed to stand at room temperature for 24 hours. The undissolved solid was recovered by filtration. It was properly dried.

The filtrate was acidified with dilute hydrochloric acid after it has been transferred into a separating funnel. The compound was extracted into dichloromethane and the solid was recovered after solvent evaporation on the water-bath. The two compounds were separated (t.l.c.). The undissolved solid was SRB_3" while the compound that went into aqueous ammonia was SRB_2.

SRB_2 (40mg) had a m.p. 290-292°C.

{Found C: 64.85, H:3.43; N: 4.64. $C_{16}H_9NO_5$ required C:65.09, H: 3.07, N: 4.74}.

M^+ 295 (100- base peak), m/e 268, 255, 240, 238, 227, 199, 171, 127, 115, 85, 69, 44, 43.

ν_{max} (Nujol) 1745cm^{-1} (-C=O), 1660, 1620 (-C=O), 1590cm^{-1} (-C=C-, aromatic), 1260, 1165, 1120, 1060cm^{-1} (ether linkages), 870, 845 and 780cm^{-1} (substituted aromatic rings).

λ_{max} ($CHCl_3$), 257(log ϵ = 4.38), 263(log ϵ = 4.44) 321 (log ϵ = 4.05) and 330nm (log ϵ = 4.05).

SRB_3" (85mg) had a m.p. 248-251°.

{Found C:64.90, H: 3.29, N: 4.74. $C_{16}H_9NO_5$ required C: 65.09, H: 3.07; N: 4.74}.

M^+ 295 (100 - base peak); m/e 267, 266, 260, 240, 239, 238 227, 199, 171, 143, 115 and 39.

ν_{max}(Nujol), 3200cm^{-1} (-OH group), 1680, 1660, 1620cm^{-1} (-C=O), 1590cm^{-1} (-C=C-, aromatics), 1210, 1160, 1100 and 1020cm^{-1} (ether linkages), 970 and 840cm^{-1} (substituted aromatic rings).

λ_{max}(MeOH 225 (log ε = 3.96, sh), 232 (log ε = 4.14), 240 (log ε = 4.16), 260 (log ε = 4.16), 310 (log ε = 4.03), 315 (log ε = 4.03) and 323 nm (log ε = 3.90, sh).

Acetylation of SRB_1 (the chromone)

SRB_1 (50mg) was dissolved in pyridine (1cc.) and acetic anhydride (1c.c.) was added. The reaction mixture was left overnight to stand at room temperature for 24 hours, after which the reaction was complete (t.l.c.). The reaction mixture was poured into water (5cm^3) and shaken properly. Saturated sodium bicarbonate solution was added to it to remove the acetic acid formed from acetic anhydride. It was extracted into chloroform. The chloroform extract was transferred to a separating funnel and treated with dilute hydrochloric acid. The chloroform layer was removed and dried with sodium sulphate, filtered

and evaporated. This gave the diacetate of SRB_1, m.p. 137-139°. M^+ 276, with other prominent peaks at m/e 234, 192, 164, 163, 124, 123, 96 and 69.

ν_{max} 1760cm^{-1} ($-OCOCH_3$), 1670, 1630cm^{-1} (-C = O)

δppm ($CDCl_3$) 2.30 (3H, s, $-OCOCH_3$); 2.40 (3H, s, $-OCOCH_3$); 6.08 (1H, s, on γ-pyrone), 6.80 (1H, d,; J = 2Hz, aromatic); 7.16 (1H, d, J = 2Hz, aromatic).

Diazomethane methylation of SRB_1.

p-Tolysulphonylmethylnitrosamide (2.15g; 0.01m) was dissolved in ether (40c.c.) cooled in ice and a solution of potassium hydroxide (0.42g; 0.0075m) in methylated spirit (10cm^3) was added. After about 5 mins., the ethereal diazomethane solution was distilled directly into the solution of SRB_1 (53mg; 0.28mM) in methanol.

After the evolution of the gas has ceased, the reaction mixture was left overnight. The reaction was clean and complete (t.l.c. plate). Evaporation of the methanol and purification on a column of silica gel gave the pure monomethylether (30mg) of SRB_1, m.p. 116-117°.

M^+ 206; prominent peaks at m/e 177, 176, 164, 149, 123, 95 and 69.

ν_{max} 1660cm^{-1}, 1620cm^{-1} (-C=O), 1580, 860, 840cm^{-1} (aromatic rings), and 1165cm^{-1} (ether linkage).

λ_{max} 210nm (log ε = 4.43), 230 (log ε = 4.34) 250 (log ε = 4.29), 275 (log ε = 4.28) and 295 (log ε = 3.7).

δppm ($CDCl_3$) 2.30 (3H, s, $-CH_3$), 3.80 (3H, s, $-OCH_3$), 6.08 (1H, s, proton at 3-position of γ-pyrone), 6.63 (2H, s, aromatic) and 12.7 (1H, s, disappeared with D_2O).

Methylation of SRB_1 with dimethylsulphate in acetone.

SRB_1 (100mg; 0.52mM), dimethyl sulphate (1cm^3; 0.01M) anhydrous potassium carbonate (500mg) and dry acetone (40cm^3) were heated under reflux for 12 hours. Removal of the solids and evaporation of the filtrate yielded a dark purple product. The product, on treatment with a solution of ammonia followed by water, gave no precipitate. It was then extracted with chloroform. Evaporation of the chloroform gave an oil which contained two compounds (t.l.c.), one faster than the starting material and the other slower. These were separated on a column of silica gel. The fast-moving compound corresponded to the monomethylether (30mg), m.p. 116-117°, while the slow-moving component

was the dimethylether (20mg), m.p. 122-123°. The spectral properties of the monomethylether remained the same. The spectral properties of the dimethylether are shown below.

δppm ($CDCl_3$) 2.30 (3H, s, $-CH_3$), 3.88 (3H, s, $-OCH_3$), 3.93 (3H, s, $-OCH_3$), 6.08 (1H, s, proton at 3-position of γ-pyrone), 6.30 (1H; d, J = 2Hz, aromatic), 6.44 (1H, d, J = 2Hz, aromatic).

Acetylation of the monomethylether of SRB_1.

The monomethylether of SRB_1 (40mg) was dissolved in pyridine (1.c.c.) and acetic anhydride (1c.c.) was added. The reaction mixture was allowed to stand at room temperature for 24 hours. The reaction mixture was poured into water (5c.c.) and saturated sodium bicarbonate solution was added and shaken properly. It was extracted with chloroform. The chloroform extract was treated with dilute hydrochloric acid to remove pyridine. The chloroform was evaporated after drying with anhydrous sodium sulphate.

Even though, from the t.l.c. plate, the product of acetylation has the same R_f value with the starting material,

the spectral properties confirmed the acetylation. After isolation and purification on a column of silica gel, the acetylated monomethylether of SRB_1 had a m.p. 150-151°.

δppm 2.30 (3H, s, $-CH_3$); 2.40 (3H, s, $-OCOCH_3$) 3.87 (3H, s, $-OCH_3$); 5.93 (1H, s, proton at 3-position of γ-pyrone), 6.51 (1H, d, J = 2Hz, aromatic), 6.68 (1H, d, J = 2Hz, aromatic).

Treatment of SRB_1 with methyl iodide and silver oxide in chloroform.

A suspension of SRB_1 (100mg; 0.52mM) in chloroform (20c.c.) was refluxed with silver oxide (200mg; 0.9mM) and methyl iodide (3c.c., 0.48M) for 10 hours. The mixture was filtered. Evaporation of the filtrate left an oily material which indicated two spots on the t.l.c. plate. These were separated on a column of silica gel. The fast-moving compound was identified as the C-alkylated monomethyl-ether of SRB_1, while the slow-moving compound was the dimethylether of the SRB_1. The C-alkylated mono ether (50mg) had a m.p. 156 - 157°, with part of it melting at 143-144°.

ν_{max} 1660, 1630cm^{-1} (-C = O), 1580, 845cm^{-1} (aromatic rings), 1175, 1160, 1130cm^{-1} (ether linkages).

δppm. 2.08 (3H, s, $-CH_3$), 2.35 (3H, s, $-CH_3$).
3.73 (3H, s, OCH_3), 6.03 (1H, s, at 3-position of γ-pyrone) 6.35 (1H, s, aromatic) and 12.7 (1H, s, disappeared with D_2O).

SYNTHESIS OF 5,7-DIHYDROXY-2-METHYL CHROMONE.

(a) Conversion of 2,4,6-trihydroxyacetophenone to 5,7-diacetoxy-3-acetyl-2-methylchromone.

A mixture of dry 2,4,6-trihydroxyacetophenone (6g; 0.036M), redistilled acetic anhydride (25cm^3; 0.19M) and freshly fused sodium acetate (10g; 0.122M) was boiled under reflux in an oil-bath at 185°C for 8 hours. The cooled, dark-brown reaction mixture was poured unto excess ice water and left in a refrigerator for 24 hours. The dry solids thus obtained were taken up in acetone solution (100cm^3) and ether was added gradually when a dark-brown, coloured, resinous solid, separated out which was filtered off. Petroleum ether (b.p. 40-60°) was added slowly to the clear solution. A further small quantity of tarry matter separated out. The pale yellow solution thus obtained gave on concentration a product (4.18g) as yellow plates and prisms. The product was purified by passing it through a

column of silica gel. Two products were obtained. The first component (1.6g) was identical with compound 135. It had a m.p. 120-122°. {Found C: 59.27, H 4.30. $C_{30}H_{28}O_4$ required C:58.82, H: 4.58}.

ν_{max} 1770cm^{-1} (-OCOCH$_3$), 1680; 1640cm^{-1} (-C=O)

δppm ($CDCl_3$), 2.24 - 2.50 (six -OCOCH$_3$ and two-CH$_3$)
6.46 (1H, d, J = 2Hz, aromatic), 6.62 (1H, d, J = 2Hz, aromatic), 6.80 (1H, d, J = 2Hz, aromatic), 7.10 (1H, d, J = 2Hz, aromatic).

The second component from the column corresponded to 5,7-diacetoxy-3-acetyl-2-methylchromone (3g), m.p. 129-130°, lit.[83], 129-131°.

ν_{max} 1760cm^{-1} (-OCOCH$_3$), 1660, 1630 (-C=O), 1600cm^{-1} (-C=C-, aromatics), 1175, 1130 and 1070cm^{-1} (ether linkage), 860 and 825cm^{-1} (substituted aromatic rings).

δppm. 2.25 (3H, s, -CH$_3$), 2.30 (6H, s, -OCOCH$_3$, -COCH$_3$) 2.48 (3H, s, -OCOCH$_3$), 6.76 (1H, d; J = 2Hz, aromatic), 7.12 (1H, d, J = 2Hz).

(b) Hydrolysis of 5,7-diacetoxy-3-acetyl-2-methylchromone.

5,7-Diacetoxy-3-acetyl-2-methylchromone (2.5g; 0.008M) was boiled with dilute hydrochloric acid (40cm^3; one part of water to two parts of acid) for thirty minutes. The product

that separated out was purified by recrystallization from acetone-pet. ether (b.p. 40-60°) when it gave 3-acetyl-5,7-dihydroxy-2-methylchromone (2g) as yellow prisms; m.p. 250-251°, lit.[84], 250-251°.

ν_{max} 1660, 1630cm^{-1} (-C=O), 1600cm^{-1} (-C=C-, aromatics), 1160, 1130, and 1070cm^{-1} (ether linkage), 940, 845cm^{-1} (substituted aromatic rings).

(c) Conversion of 3-acetyl-5,7-dihydroxy-2-methylchromone to 5,7-dihydroxy-2-methylchromone.

The 3-acetyl derivative (1.5g; 0.0064M) was digested in aqueous sodium carbonate solution (20cm^3, 10%) by boiling under reflux for 2 hrs. Acidification of the cooled reaction mixture gave 5,7-dihydroxy-2-methylchromone (1g), which appeared as colourless plates and needles after passing it through a column of silica gel; m.p. 280-282°, lit. value[84], 281-282°C.

M^+ m/e 192. ν_{max} 1660, 1630cm^{-1} (-C=O), 1600cm^{-1} (-C=C-, aromatic), 1070, 1020cm^{-1} (ether linkage), 850 and 820cm^{-1} (substituted atomatic ring).

Acetylation of SRB_4.

SRB_4 (60mg; 0.2mM) was dissolved in pyridine ($1cm^3$) and acetic anhydride ($1cm^3$) added. The reaction mixture was left for 48 hours. The t.l.c. plate indicated three components, a fast-moving component, a slow-moving component and the third component has R_f value close to that of the starting material. Methanol ($15cm^3$) was added to the reaction mixture to destroy the acetic anhydride. The methanol was completely removed on the water-bath. Dilute hydrochloric acid was added to the material left behind and extracted into chloroform. The chloroform extract was dried with anhydrous magnesium sulphate and evaporated. The mixture was separated on a column of silica gel. Because of the close R_f values, separation was difficult. The fast-moving component (8mg) came down as a mixture (that is, with a little of the diacetate). The diacetate of SRB_4 was obtained pure (30mg), m.p. 217-219°C.

ν_{max} $3150cm^{-1}$ (-N-H), $1750cm^{-1}$ ($-OCOCH_3$), 1660, $1630cm^{-1}$ (-C=O), 1600, 850, and $750cm^{-1}$ (substituted aromatic rings).

δppm. 2.05 (3H, s, $-OCOCH_3$); 2.32 (3H, s, $-CH_3$); 2.36 (3H, s, $-OCOCH_3$); 3 - 3.8 (6H, m, methylene and methine);

6.02 (1H, s, at 3-position of γ-pyrone),
6.60 (1H, s, aromatic), 6.93 (1H, d, J = 4Hz,
at the base of an acetoxyl group).

Amide and monoacetate of SRB_4

SRB_4 (50mg; 0.16mM) was dissolved in acetic acid ($1cm^3$) followed by the addition of acetic anhydride ($1cm^3$) and p-toluenesulphonic acid (1mg) in catalytic amount. The reaction mixture was left overnight. The reaction mixture was later transferred into a separating funnel and treated with water (distilled). After shaking for sometimes, it was basified with sodium bicarbonate and extracted into chloroform. The chloroform layer was dried with anhydrous $MgSO_4$ and evaporated on a water-bath.

The NMR confirmed it to be the amide of the monoacetate of SRB_4. On passing it through a column of silica gel, the amide was almost half-converted to the monoacetate, and when recrystallized, the whole product was converted to the monoacetate (40mg), m.p. 153-155°C. M^+ 359 (15%), m/e 296 (23%); 295 (85%); 294 (17%); 261 (35%), 243 (16%), 242 (44%), 192 (30%), 100 (9%), 69(11%), and 43 (100%).

δppm 2.02 (6H, s, - $OCOCH_3$);

2.32 (3H, s, $-CH_3$); 2.8 - 3.7 (6H, m, methylene and methine); 6.05 (1H, s, on γ-pyrone) 6.24 (1H, s, aromatic); 6.84 (1H, d; J = 4Hz, proton at the base of acetoxyl group) and 12.5 (1H, s, disappeared with D_2O).

Methylation of SRB_4

A suspension of SRB_4 (70mg; 0.22mM) in chloroform ($20cm^3$) was refluxed with silver oxide (150mg; 0.65mM) and methyl iodide ($2.5cm^3$; 0.04M) for 6 hours. The mixture was filtered and evaporated. The oily material left behind contained three compounds. The compound recovered from the column of silica gel which has almost the same R_f value with the starting material proved to be the dimethylether. The dimethylether (20mg) recovered has a m.p. 225-226°.

δppm 2.30 (3H, s, $-CH_3$); 3-3.8 (6H, m; methylene and methine);

3.40 (3H, s, - OCH_3);

3.90 (3H, s, $-OCH_3$); 5.68 (1H, d, J = 4Hz, at the base of a methoxyl group);

6.01 (1H, s, on γ-pyrone) and 6.35 (1H, s, aromatic).

Dehydrogenation of SRB_4

A mixture of SRB_4 (105mg; 0.33mM), palladium on carbon (10mg) and nitrobenzene ($5cm^3$) was placed in a 25ml-round-bottomed flask and heated under reflux for 3 hours. The hot mixture was filtered by suction, and the filtrate was allowed to cool. The solvent (i.e. nitrobenzene) was removed under reduced pressure. The solid recovered was passed through a column of silica gel. The needle-like crystals were not soluble in chloroform. The product of dehydrogenation (20mg), has a m.p. 294-296°C.

M^+ 295 (100%), m/e 267, 255, 238, 192, 172, 134, 105, 100, 92, 83, and 69.

ν_{max} $1740cm^{-1}$ (-CO); $1660cm^{-1}$ (-C=O); 1595cm (-C=C-, aromatic), 1160 and $1165cm^{-1}$ (ether linkages)

λ_{max} (in nm) (log ε) 258 (4.38), 265 (4.41), 269 (4.41), 321 (4.06) and 328 (4.07).

Acetylation of the dehydrogenation product.

Dehydrogenation product (8mg; 0.028mM) was dissolved in pyridine ($0.5cm^3$), followed by the addition of acetic anhydride ($0.5cm^3$) and was heated at 100°C for 6 hours. On cooling the flask for a few hours some white crystals

separated out from the reaction mixture. The crystals were collected by filtration. The product of acetylation (4mg) has a m.p. 285-287° (subl.). The molecular ion appeared at M^+ 295. This was interpreted to mean the loss of the acetyl group at such a high temperature.

ν_{max} 1750cm^{-1} ($-OCOCH_3$), 1660cm^{-1} ($-C=O$), 1595cm^{-1} ($-C=C-$, aromatic), 1260, 1170, 1110 and 1060cm^{-1} (ether linkages) and 870, 850 and 835cm^{-1} (substituted aromatic rings).

Alkaline hydrolysis of SRB_4

SRB_4 (100mg; 0.32mM) was dissolved in a solution of potassium hydroxide pellets (1gm) in methanol (20cm^3). The mixture was refluxed for 8 hours. The solvent was completely removed by evaporation and the residue acidified with dilute hydrochloric acid. It was then extracted with chloroform. The t.l.c. plate showed one major component and another minor one with a little of the starting material.

Separation on a column of silica gel afforded a pure compound (22mg); m.p. 273-274°C, and the other component, apart from being too small could not be

isolated. The major product of hydrolysis which was identical with noreugenin has the molecular ion, M^+ as 192.

ν_{max} 1660, 1620cm^{-1} (-C=O), 1165cm^{-1} (ether linkage); 1570cm^{-1} (-C=C-, aromatic).

Acetylation of SRB_3.

SRB_3 (50mg; 0.15mM) was dissolved in pyridine (1cm^3) and acetic anhydride (1cm^3) was added. The reaction after 24 hours was not complete (t.l.c.). It was then allowed to stand for another 24 hours, after which the reaction was complete (t.l.c.).

Methanol (15cm^3) was added to the reaction mixture to destroy the acetic anhydride. The methanol was distilled off completely on a water-bath. Dilute hydrochloric acid was added to the material left behind and extracted with chloroform. The chloroform extract was dried with anhydrous $MgSO_4$. Evaporation of the chloroform left a product, which solidified on standing for a few hours. Purification on a column of silica gel yielded a diacetate of SRB_3, m.p. 223 - 225°C.

ν_{max} 1750cm^{-1} (-OCOCH$_3$), 1660 and 1620cm^{-1} (-C=O)

δppm 2.01 (3H, s, -OCOCH$_3$), 2.38 (3H, s, -CH$_3$),
2.40 (3H, s, -OCOCH$_3$), 2.95 (3H, s, -N-CH$_3$).
3 - 3.8 (6H, m, methylene and methine),

6.06 (1H, s, on γ-pyrone), 6.53 (1H, s, aromatic),
6.95 (1H, d, J = 4Hz at the base of an acetoxyl group).

Methylation of SRB_3

A solution of SRB_3 (50mg; 0.15mM) in chloroform ($20cm^3$) was shaken vigorously with silver oxide (100mg; 0.43mM) and methyl iodide ($2cm^3$; 0.032m) for 1 hour. Two further additions of silver oxide (50mg) and methyl iodide ($1cm^3$) were made at intervals of one hour and shaking continued but the reaction was not complete.

The reaction mixture was transferred to the water-bath and refluxed for 5 hours. The mixture was filtered and evaporated. An oily material was recovered which was passed through a column of silica gel for purification. The oily diether (27mg) of SRB_3, which later crystallized had a m.p. 169-172°.

δppm 2.35 (3H, s, $-CH_3$); 2.93 (3H, s, $-N-CH_3$);
3.48 (3H, s, $-OCH_3$); 3.93 (3H, s, $-OCH_3$);
3 - 3.8 (6H, m, methylene and methine);
5.74 (1H, d; J = 4Hz, at the base of a methoxyl group);
6.05 (1H, s, on γ-pyrone) 6.40 (1H, s, aromatic).

Alkaline hydrolysis of SRB_3

SRB_3 (60mg; 0.18mM) was dissolved in a solution of potassium hydroxide pellets (0.5g) in methanol ($10cm^3$). The mixture was refluxed for 8 hours.

The solvent was completely removed by evaporation and the residue acidified with dilute hydrochloric acid. It was then extracted with chloroform. The t.l.c. plate showed that the reaction was complete but the other fragment containing nitrogen remained in the aqueous acidic layer. Purification of the recovered material gave a compound (10mg) which was identical with noreugenin, with m.p. 265-267°C (decomp.) (recryst. from $MeOH/CHCl_3$).

ν_{max} 1660 and 1620cm^{-1} (-C=O), 1560, 1500cm^{-1} (substituted benzene ring), 1165cm^{-1} (ether linkage).

λ_{max}(nm), 214, 228, 251, 257, 295 and 325.

δppm. 2.27 (3H, s, $-CH_3$), 6.08 (1H, s, on γ-pyrone)
6.18 (1H, d, J = 2Hz, aromatic);
6.28 (1H, d, J = 2Hz, aromatic) and
12.7 (1H, s, disappeared with D_2O).

Dehydration of SRB_3.

SRB_3 (30mg; 0.09mM) was dissolved in dry benzene ($15cm^3$). p-Toluenesulphonic acid (8mg; 0.042mM) was added and the mixture refluxed for 10 hours (followed up with t.l.c. plate). The reaction was not complete after 10 hours. The reaction mixture was allowed to cool, when further attempt to improve it failed. It was washed with saturated sodium bicarbonate solution, water, and dried with anhydrous magnesium sulphate. Evaporation of benzene under reduced pressure gave an impure product (10mg). Since separation could not be effected no good spectra could be obtained.

ATTEMPTED SYNTHESIS OF THE DEHYDROGENATION PRODUCT

(a) Acylation of 5,7-dihydroxy-2-methylchromone with nicotinyl chloride.

In a $100cm^3$ two-necked flask fitted with a mechanical stirrer and a dropping funnel was placed nicotinic acid (2.5g; 0.02M). The stirrer was started and redistilled thionyl chloride ($10.5cm^3$, or 16.6g; 0.14M) was added in a slow stream over a period of 15min. After the addition was complete, the dropping funnel was replaced with a reflux condenser protected

with a calcium chloride tube and the mixture was heated on the steam-bath with continuous stirring for 1 hour. Then the reflux condenser was replaced by one set for downward distillation and the excess thionyl chloride was removed by distillation at reduced pressure as heating on the steam-bath was continued. After most of the thionyl chloride has been distilled, 5,7-dihydroxy-2-methylchromone (2g; 0.01M) was introduced. The flask was fitted with a reflux condenser and placed in an ice-salt bath. The stirrer was started and anhydrous aluminium chloride (6.68g; 0.05mole) was added in portions over a period of 30 mins. The ice-bath was removed and the flask was allowed to warm to room-temperature and was finally heated under reflux for 6 hours.

The dark-brown reaction mixture was cautiously poured into a mixture of ice/conc. HCl ($40cm^3$). The organic layer was separated and discarded. The acid solution was extracted with ether which was discarded, then it was treated with aqueous ammonia until strongly alkaline. The organic material was extracted into chloroform. The chloroform extract was washed with water

and dried with anhydrous $MgSO_4$. The chloroform was removed by distillation on a water-bath. The product proved to be the starting material in all respects (t.l.c., m.pt. and infrared spectra).

(b) Preparation of oxalyl chloride

Powdered anhydrous oxalic acid (4.5g; 0.05M) and phosphorus pentachloride (20.8g; 0.1M) were mixed together properly while cooling in an ice-bath continued. Then the ice-bath was removed and allowed to warm up at room temperature. It was left for 24 hours at room temperature. At this time the reaction mixture has turned to a liquid.

It was then fractionally distilled and the fraction that came out between 60° and 100°C was collected. The fraction collected was fractional distilled again and the fraction that boiled between 63 - 65°C, lit.[89] 63.5 - 64°C, was collected. The yield of the oxalyl chloride was 3g. (i.e. $2cm^3$).

Preparation of nicotinic anhydride by the reaction of oxalyl chloride with potassium nicotinate.

To a suspension of potassium nicotinate (1.29g; 0.008M) which had been ground into fine particles and dried at 135°, in anhydrous benzene ($5cm^3$) was added with mechanical

stirring and cooling in an ice-bath, oxalyl chloride (0.5g; 0.004M) in anhydrous benzene ($3cm^3$) during 20 minutes. The cooling bath was removed after another 15 mins. and the suspension stirred at room temperature for one hour; then at the refluxing temperature for another 1 hour. It was filtered hot. The filtrate without further concentration was left at room temperature during when the anhydride crystallized out, and later filtered to give nicotinic anhydride (300mg); m.p. 120-121°, (lit.[98] 122.5 - 123.5°; lit.[88] 123-126°.).

Acylation of 5,7-dimethoxy-2-methylchromone with nicotinic anhydride.

In a $100cm^3$ two-necked flask fitted with a mechanical stirrer and a dropping funnel was placed a mixture of nicotinic anhydride (200mg; 0.001M) and aluminium chloride (270mg; 0.002m). The flask was put in an ice-bath to maintain the temperature between 5° and 10°. The stirrer was started and 5,7-dimethoxy-2-methylchromone (220mg; 0.001M) which was dissolved in redistilled nitromethane was added dropwisely. After the addition was complete, the dropping funnel was replaced by a stopper. The flask was allowed to remain in the ice-bath for another 30 mins., then the ice-bath was removed

and the flask was allowed to warm to room tempt. The flask was finally heated at 60°C for 5 hours.

The dark-brown reaction mixture was cautiously poured onto a mixture of ice and conc. HCl. The compound was extracted into chloroform. Removal of the chloroform on a water-bath left behind liquid product which gave fine white crystals when cooled. The crystals were filtered off and properly dried. The product (20mg), has a m.p. 42°. The molecular ion was given as M^+ 236.

ν_{max} 3400cm^{-1} (-OH), 1660, 1620cm^{-1} (carbonyl); 1580cm^{-1} (-C=C-, aromatic); 1160, 1120 and 1090cm^{-1} (ether linkages).

(c) SYNTHESIS OF 4-HYDROXYNICOTINIC ACID.

(i) Preparation of 3-methyl-4-nitropyridine-1-oxide

Aqueous hydrogen peroxide (80cm^3, 30%) was added to a stirred solution of 3-methylpyridine (50g; 0.54M) in glacial acetic acid (150cm^3; 2.6M) during 30min; the tempt. being kept below 10°. After being heated at 70° for 24 hours, the solution was evaporated under reduced pressure. The resulting pale yellow viscous oil was cooled below 5° and a mixture of concd. H_2SO_4 (158cm^3) and concd. nitric acid (124cm^3) was added dropwise with vigorous

stirring. After being heated cautiously to 100-105° for 4 hrs., the mixture was poured on crushed ice and the pH adjusted to 3 (Na_2CO_3). The solid was filtered off and extracted with hot acetone. This extract was combined with material obtained by extracting the filtrate with chloroform. After removal of solvents under reduced pressure the product was crystallized from acetone/$CHCl_3$. The yield of 3-methyl-4-nitropyridine-1-oxide, m.p. 135-137° (lit. value[92]; 136-138°), was 12g.

(ii) Preparation of 4-chloronicotinic acid.

3-Methyl-4-nitropyridine-1-oxide (25g; 0.18M) was dissolved in chloroform (500cm^3) and the solution was saturated with dry HCl at room tempt. Phosphorus trichloride (40cm^3; 0.46M) was added dropwise to the stirred solution (at 0-5°C). After the solution had been allowed to reach room tempt., reaction was intiated by cautious warming on a steam-bath. The reaction then proceeded without heating and was moderated as necessary by cooling. When the reaction subsided the mixture was heated under reflux for 30mins. and then evaporated under reduced pressure. The residue was dissolved in iced water (400cm^3) and after

the addition of an excess of saturated aqueous sodium carbonate the solution was steam-distilled giving 4-chloropicoline ($16cm^3$; 18.5g; d:1.16g/c.c.).

This was dispersed in water ($250cm^3$) and potassium permanganate (59.5g; 0.38M) was added. The stirred mixture was heated at 80-90° for 4hrs. and then steam-distilled to remove any unchanged chloropicoline. The precipitated manganese dioxide was filtered off, and washed well with hot water. After the filtrate had been concentrated to about $50cm^3$, the pH was adjusted to 3 with concd. HCl. The precipitated 4-chloronicotinic acid was quickly filtered off and pressed as dry as possible before being washed with acetone ($3x\ 30cm^3$) and then with dry ether. The yield was 12g. A specimen of the acid was purified by dissolution in theoretical amount of N-sodium hydroxide solution and reprecipitation by slow addition of an equivalent of dilute HCl. The acid formed prismatic needles. decomp. 174-176° lit.[93] m.p. 162-163°, lit.[94] 164°).

ν_{max} $3220cm^{-1}$ (-OH), $1725cm^{-1}$ and $1630cm^{-1}$ (-C=O).

(iii) Preparation of 4-hydroxynicotinic acid.

4-chloronicotinic acid (3g; 0.019M) was heated for 1hr. in water ($60cm^3$). After adjusting the pH of the solution to 4 with NaOH, evaporation to half bulk and cooling afforded the hydroxyl acid (2.2g), m.p. 249-250°. (Lit.[91], m.p. 250°), 260° (decomp.).

The molecular ion, M^+ was given as 139 which was in agreement with the expected molecular ion.

ν_{max}: 3250cm^{-1}, 3100cm^{-1} (-OH), 1750cm^{-1} and 1700 cm^{-1} (C=O).

Preparation of β-cyanopyridine.

Nicotinamide (25g; 0.205M) was mixed with phosphorus pentoxide (30g; 0.21M) in a 250ml round-bottomed flask. The flask was immersed in an oil-bath and the content distilled under reduced pressure of about 20mm. The temperature of the oil-bath was raised rapidly to 280°C. The nitrile crystallized on cooling to a snow-white solid. This gave pure needle-like crystals (15g) from a mixture of chloroform and pet. ether (40-60°), m.p. 49-50°,(lit.[97], 49°, lit.[99], 50-51°).

Preparation of Anhydous Zinc Chloride.

Finely ground zinc chloride hydrate or the wet anhydous zinc chloride (20g) was put into a round-bottomed flask, and freshly distilled thionyl chloride ($50cm^3$, ca 0.64M) was added at room temperature.[100] Evolution of sulphur dioxide and hydrogen chloride began at once. After the evolution, the slurry was refluxed for 2 hours and distilled. The excess thionyl chloride was removed under reduced pressure. The solid left behind was transferred immediately to a vacuum desiccator containing potassium hydroxide and stored for 18 hours to removed the remaining thionyl chloride. The anhydrous zinc chloride (15g) was pure and dry enough for any synthetic work.

Synthesis of 2,4,6-trihydroxynicotinophenone.

A mixture of well-dried phloroglucinol (2.52; 0.02M), nicotinonitrile or β-cyanopyridine (4.16; 0.04M), finely powdered freshly fused zinc chloride (1g) in 1,2-dimethoxyethane ($20cm^3$) was put in a 250ml-flask. The flask was cooled in an ice-salt mixture and shaken occasionally while a rapid stream of dry hydrogen chloride

was passed through the solution for two hours. The flask was allowed to stand in a ice-chest for 24 hours and hydrogen chloride was again passed into the mixture for two hours. The flask was stoppered and allowed to stand in a refrigerator for three days.

The bulky orange-yellow precipitate of the ketimine hydrochloride was separated by decanting the solvent. The solid was transferred to a 250ml round-bottomed flask with 100cm^3 of hot water. The solution was refluxed for two hours. About 1gm of decolorizing charcoal was added and the solution was boiled for another five minutes and filtered hot. The decolorizing charcoal was washed with two 10cm^3 portions of boiling water and this filtrate was added to the main portion.

After standing overnight, yellow prisms of 2,4,6-trihydroxynicotinophenone (3g) were obtained. The product on recrystallization by dissolving in dilute sodium hydroxide, and acidifying to a pH of 3.0 gave pure compound (2.8g), m.p. 253-255°.

IR: ν_{max} 3450cm^{-1}, 3230cm^{-1} (-OH), 1640cm^{-1} (-CO)

UV: λ_{max} 217, 221, 305 and 315nm

Triacetate; m.p. 210-211°.

NMR of triacetate: δppm 1.92 (6H, s, two-OCOCH$_3$), 2.30 (3H, s, -OCOCH$_3$), 6.92 (2H, s, aromatics), 7.38, 7.93, 8.67 and 8.88 (four pyridine protons).

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